SUPPLEMENT No. 2

THE JOURNAL
OF
EXPERIMENTAL MEDICINE

EDITED BY
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VOLUME XLVIII, No. 2 AUGUST 1, 1928

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By HIDEYO NOGUCHI, M.D.

Entered as second-class matter June 3, 1916, at the Post Office at Baltimore, Md., under the act of March 3, 1897.
Accepted for mailing at special rate of postage provided for in section 1103, act of October 3, 1917.
Authorized June 29, 1918.

Made in the United States of America

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NEW YORK
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
1928

WAVERLY PRESS, INC.
The Williams & Wilkins Company
Baltimore, U. S. A.

The Journal of Experimental Medicine, August 1, 1928,
Vol. xlviii, No. 2. Supplement No. 2.

THE ETIOLOGY OF TRACHOMA.

By HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 1 to 31.

(Received for publication, October 27, 1927.)

PART I.

PATHOLOGICAL, BACTERIOLOGICAL, AND DIRECT INOCULATION INVESTIGATIONS OF THE TRACHOMA PREVAILING AMONG AMERICAN INDIANS.

The incitant of trachoma is still unestablished. Most investigators are of the opinion that trachoma is a distinct disease induced by a specific microorganism or virus as yet unrecognized. A few consider it due to repeated infections with one or more varieties of known pathogenic bacteria.[1] Still others believe that defective diet is a factor in its causation.[2] Each of these views being well within the range of possibility, the search for the incitant must be based upon the broadest possible conceptions. I shall, however, confine myself here chiefly to the subject of the microorganisms which may be concerned in the production of the disease.

Up to the present no microorganism has been obtained in pure culture which can be found constantly and exclusively in trachomatous lesions and is capable of reproducing in man or animals the clinical and anatomical changes characteristic of trachoma. The minute bacillus found by Koch[3] (1883) to be associated with Egyptian ophthalmia was shown by Weeks[4] (1885) to be the incitant of an acute conjunctivitis ("Koch-Weeks conjunctivitis"). A bacillus was isolated by L. Müller[5] from cases of acute conjunctivitis which he believed to be trachoma,

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[1] Williams, A. W., *Collected Studies Bureau Lab., New York City Dept. Health*, 1912–13, vii, 159; *Arch. f. Ophth.*, 1913, xlii, 506.

[2] Royer, B. F., *J. Am. Med. Assn.*, 1926, lxxxvii, 482.

[3] Koch, R., *Wien. med. Woch.*, 1883, xxxiii, 1550.

[4] Weeks, J. E., *New York Med. Rec.*, 1887, xxxi, 571.

[5] Müller, L., *Wien. klin. Woch.*, 1897, x, 920; *Arch. Augenheilk.*, 1900, xl, 13.

but later studies, particularly by Knapp[6] (1904), showed that Müller's microorganism belongs to the group of the influenza bacillus, to which the Koch-Weeks bacillus is also related. Most of the microorganisms obtained by early investigators (Sattler,[7] Michel,[8] Rählmann,[9] Poncet,[10] etc.) were cocci, some Gram-positive. Noiszewski's *Microsporon trachomatosum*[11] was a mold, isolated on gelatin from the eye of a calf. Cazalis's *Streptothrix försteri*[12] is of uncertain nature. Burchardt's coccidium,[13] and the blastomyces described by various authors[14] (Gonella, Guarnieri, Lodato, Addario, Stiel) recall the peculiar round or oval degenerative bodies so commonly found in Leber's epithelioid cells. None of these organisms produced trachoma, either in experimental animals or human volunteers.

A new era in the study of the etiology of trachoma opened in 1907, when Halberstädter and Prowazek[15] described characteristic intracellular inclusions occurring in the conjunctival epithelial cells in cases of trachoma in Java, while independently in the same year Greeff, Frosch, and Clausen[16] detected in trachomatous materials studied in Berlin, Posen, and East Prussia minute, Gram-negative diplobacteria which they later concluded were identical with Halberstädter and Prowazek's trachoma bodies. The pathogenic nature of the inclusion bodies, which were designated chlamydozoa, was inferred by Halberstädter and Prowazek, who produced a mild form of conjunctivitis by inoculating the eyes of ourang-utans with materials from human cases, and who determined the presence of the cell inclusions in the experimental disease. The follicles which are so characteristic of trachoma did not, however, appear in the animals. The problem was soon complicated, however, by the finding that similar bodies were associated with various forms of non-trachomatous conjunctivitis (blennorrhea of the new-born, of

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[6] Knapp, A., *Studies from the Pathological Laboratory, Columbia University*, 1901–04, ix, No. 13.

[7] Sattler, H., *Ber. Versamml. ophth. Ges.*, 1881, xiii, 18.

[8] Michel, J., *Arch. Augenheilk.*, 1885–86, xvi, 348.

[9] Rählmann, E., *Beitr. Augenheilk.*, 1905, vii, No. 62, 35.

[10] Poncet, F., *Bull. et mém. Soc. franç. ophth.*, 1886, iv, 158.

[11] Noiszewski, K., *Centr. prakt. Augenheilk.*, 1891, xv, 65.

[12] Cazalis, Thèse de Montpellier, 1896.

[13] Burchardt, *Centr. prakt. Augenheilk.*, 1897, xxi, 33.

[14] Gonella, *Communic. fatta 10. Soc. Scienz. med. N. Cagliari*, Genoa, 1896. Guarnieri, G., *Clin. mod.*, 1896, ii, 290. Lodato, G., *Arch. ottal.*, 1908–09, xvi, 49. Addario, C., *Arch. Augenheilk.*, 1909, lxiv, 265. Stiel, A., *Deutsch. med. Woch.*, 1912, xxxviii, 2369.

[15] Halberstädter, L., and von Prowazek, S., *Deutsch. med. Woch.*, 1907, xxxiii, 1285; *Arch. Protistenk.*, 1907, x, 335; *Arb. k. Gsndhtsamte*, 1907, xxvi, 1.

[16] Greeff, R., Frosch, and Clausen, W., *Arch. Augenheilk.*, 1907, lviii, 52; 1907–08, lix, 203. Clausen, W., *Klin. Jahrb.*, 1908, xix, 101.

gonorrheal or other origin, and Koch-Weeks conjunctivitis), as well as with chronic urethritis. Prowazek and Halberstädter thought that the inclusions represented different varieties of chlamydozoa and were responsible for the morbid conditions with which they were associated, and Lindner[17] believed that the presence of the chlamydozoa indicated trachoma, irrespective of the clinical picture. On the other hand, Williams[1] regarded the inclusions as degenerated bacteria, and Noguchi and Cohen[18] were inclined to consider them as independent pathogenic microorganisms which may induce a pure infection of the conjunctivæ or other mucosa, or may be associated with other organisms in mixed infections. The latter authors found that intratesticular inoculation of rabbits with the Koch-Weeks bacillus produced cell inclusions closely resembling those found in the conjunctival epithelial cells during the subacute stage of Koch-Weeks conjunctivitis. They also obtained in culture a microorganism which resembled somewhat the inclusion bodies in morphology but was non-pathogenic.

An important point in this connection was brought out by Fritsch, Hoffstädter, and Lindner,[19] who found that materials derived from cases of non-gonorrheal blennorrhea of the new-born produced in baboons a granular conjunctivitis of chronic nature in which inclusion bodies were present during the period of acute inflammation, before the follicles appeared. A baboon inoculated into the urethra with materials from human trachoma showed no reaction.

In human trachoma the inclusion bodies are demonstrable only during the acute stage of the disease. But in view of the fact that they occur in other conditions than trachoma, their presence does not necessarily indicate a trachomatous condition, nor does their absence exclude trachoma.

A real step forward was taken in 1905, when Hess and Römer[20] succeeded in transmitting a trachomatous disease to two baboons, and Morax[21] produced a similar condition in a chimpanzee, by inoculation of human materials. The baboons were partially susceptible only; the lesions produced were less extensive than those in man and underwent retrogression within 2 to 3 months without scar formation or involvement of the cornea. In histology, however, the lesions closely reproduced those of early trachoma. Transfer of the lesions to another baboon succeeded with material removed 3 weeks after inoculation; while material taken 2 months after inoculation was ineffective. The experiments of Morax showed that chimpanzees are only occasionally susceptible to trachoma, since only

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[17] Lindner, K., *Berl. klin. Woch.*, 1909, xlvi, 2277; *Wien. klin. Woch.*, 1909, xxii, 1555, 1659, 1697, 1742; 1910, xxiii, 283; *Arch. f. Ophth.*, 1910, lxxvi, 559; 1911, lxxviii, 345; 1913, lxxxiv, 1; *Deutsch. med. Woch.*, 1910, xxxvi, 1326.

[18] Noguchi, H., and Cohen, M., *J. Exp. Med.*, 1913, xviii, 572.

[19] Fritsch, H., Hoffstädter, A., and Lindner, K., *Arch. f. Ophth.*, 1910, lxxvi, 547.

[20] Hess and Römer, *Arch. Augenheilk.*, 1906, lv, 1.

[21] Morax, V., *Ann. ocul.*, 1911, cxlv, 414.

one of eight inoculated with the materials derived from six cases became infected. The lesions in this animal developed in about 11 days and reached their maximum in 30 days. When death occurred, 39 days after inoculation, the lesions were already in retrogression. Histological study of the lesions removed 31 days after inoculation revealed numerous follicles and lymphocytic infiltration of the subepithelial layer of the cul-de-sac and upper part of the tarsal conjunctiva. Transfers made to a chimpanzee and a *Macacus* with the affected tissues yielded no result. In the same year Morax inoculated unsuccessfully an ourang-utan and three baboons with human trachomatous materials.

Hess and Römer's further experiments with thirteen baboons showed that this species is rather resistant to trachoma. A baboon inoculated with a 3 weeks old lesion excised from another gave a positive result, but a second inoculated with a 2 months old lesion from the same animal failed to react. Uniformly negative results were recorded[22] by Heymann with three baboons, Addario la Ferla with two, Herford with two, Flemming with one, and Fritsch, Hoffstädter, and Lindner[19] with one. Axenfeld and Verderame[23] reported mild conjunctivitis and a few discrete follicles in two of four inoculated baboons and Greeff[24] and di Santo[25] in one of three, while Löhlein and Botteri[26] observed more numerous follicles in two of three of such animals. The lesions in the last instances endured about 2 months only and disappeared without scar or pannus formation. Böing[27] described a peculiar acute conjunctivitis developing within 4 days and lasting 6 weeks in one of two baboons inoculated. No follicles arose, but after 8 weeks the conjunctiva showed scars.

Nicolle, Cuénod, and Blaizot[28] have been more successful in the reproduction of trachomatous lesions by inoculation of human material into various species

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[22] Morax, V., Lindner, K., and Bollack, *Ann. ocul.*, 1911, cxliv, 321. Addario la Ferla, *Ann. ottal.*, 1912, xli, 278. Heymann, B., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, viii, 623. Herford, *Zentr. prakt. Augenheilk.*, 1908, xlvii, 206; *Klin. Monatsbl. Augenheilk.*, 1909, xlvii, 225. Flemming, *Arch. Augenheilk.*, 1910, lxvi, 63.

[23] Axenfeld, T., Ætiologie des Trachoms, Jena, 1914; Lehrbuch und Atlas der Augenheilkunde, Jena, 7th edition, 1923.

[24] Greeff, R., *Klin. Jahrb.*, 1909, xxi, 452; *Arch. Augenheilk.*, 1907–08, lix, 203.

[25] di Santo, C., *Klin. Jahrb.*, 1909, xxi, 491.

[26] Löhlein, W., *Arch. Augen heilk.*, 1911, lxx, 392. Botteri, A., *Klin. Monatsbl. Augenheilk.*, 1912, i, 653.

[27] Böing, W., *Arb. k. Gsndhtsamte*, 1912, xl, 235.

[28] Nicolle, C., Cuénod, A., and Blaizot, L., *Compt. rend. Acad.*, 1911, clii, 1504; 1912, clv, 241; 1913, clvi, 1177; *Arch. Inst. Pasteur Tunis*, 1911, No. 3, 185; 1913, 157. Nicolle, C., and Cuénod, A., *Compt. rend. Acad.*, 1908, cxliv, 1001; *Arch. Inst. Pasteur Tunis*, 1909, iii, 149; *Arch. Inst. Pasteur Afrique Nord*, 1921, i, 149. Nicolle, C., and Lumbroso, U., *Arch. Inst. Pasteur Tunis*, 1926, xv, 240.

of monkeys. Five chimpanzees developed a chronic, non-inflammatory, granular conjunctivitis, in two of which the condition persisted for more than a year. In respect to severity and involvement of the tarsal conjunctiva the condition resembled closely human trachoma; sclerotic changes occurred in some instances. These investigators found *Macacus inuus* susceptible to trachoma and *Macacus rhesus* and *Macacus sinicus* much less so. Moreover, they state that rabbits appear susceptible and that the virus is transferable from them to monkeys a year after injection into the rabbit conjunctiva. However, a difficulty in recognizing the true trachoma virus in rabbits arises from the presence in Tunis of a rabbit virus which induces granular conjunctivitis. This spontaneous conjunctivitis of the rabbit is transferable also to certain monkeys. On the other hand, Nicolle and his coworkers succeeded in maintaining the trachoma virus by passages from chimpanzee to magot, magot to magot, and magot to chimpanzee, rabbits being also occasionally used in the series.

The number of other monkeys—*Macacus rhesus*, *M. nemestrinus*, *M. cynomolgus*, and *Cercopithecus*—used for transmission experiments on trachoma is rather small. In general these animals proved rather resistant to inoculation. Bajardi,[29] however, reports the production of mild but definite trachomatous lesions in three *Macacus* (species?), which developed within 2 weeks, and reached their maximum in 7 to 8 weeks. Bertarelli and Cecchetto[30] reproduced the complete picture of granular trachoma in a *Macacus* (species?), the lesions healing with scars in 9 months.

To sum up, then, no apes or monkeys have been found to be uniformly susceptible to trachoma, though sometimes typical lesions have been reproduced in chimpanzees, magots, and certain *Macacus* monkeys. Usually these lesions receded within 2 to 3 months, but occasionally they persisted for months; scars seldom followed healing. Pannus has never been observed in monkeys, though Nicolle and his collaborators occasionally observed keratitis in magots. The experiments are, however, extremely important, because they furnish data on the infectiousness of trachoma and the susceptibility of animals and their reactions to inoculation of materials from human trachoma, and for comparison with experiments dealing with the effects of inoculation of cultures obtained from human trachomatous lesions.

The only reliable knowledge we possess regarding the inciting agent of trachoma is that it occurs in the trachomatous lesions and is capable of being transmitted to certain species of animals. Microscopic examination of the affected tissues has seldom been convincing as

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[29] Bajardi, P., *Gior. r. Accad. med. Torino*, 1906, xii, series 4, 385; *Clin. ocul.*, 1907, viii, 2719.

[30] Bertarelli, E., and Cecchetto, E., *Centr. Bakt.*, *1. Abt.*, *Orig.*, 1909, l, 36; 1908, xlvii, 432.

regards this point, since it is rare that morphological features and staining reactions suffice of themselves to differentiate specific from associated non-pathogenic organisms. Furthermore, the number of microorganisms actually present in the lesions might be too small to attract notice. In cultivation experiments the possibility of identifying the specific microorganism is greater, provided the conditions of cultivation—types of culture media, temperature—are sufficiently varied; but the chance of dismissing the right organism as a known pathogen or a saprophyte is very great unless provisions are made to test every microorganism cultivated for its effect on susceptible animals. The choice of experimental animal and the availability of animals in sufficient numbers are of the greatest importance.

In our studies the following plan was carried out:

1. The materials employed were derived from well advanced, untreated cases of human trachoma.
2. The number of cases selected was small, in order that each might be studied in minutest detail.
3. Direct inoculation of monkeys was made with fresh material.
4. The cultures were kept at low temperatures (15–30°C.) as well as at 37°C., recent experience with other parasites (leptospiras, *Bartonella*, herpetomonads) having shown that body temperature may on occasion be detrimental to growth in cultures.
5. Several special culture media were employed, including the semisolid leptospira medium,[31] which had proven so successful in the cultivation of certain human parasites, blood agar plates of various kinds, Avery plates,[32] and the solid ascites-agar medium which had been used for the cultivation of anaerobic microorganisms.[33]
6. The culture plates and tubes were examined at frequent intervals for several weeks after implantation, and each microorganism obtained was isolated.
7. The criterion chosen for the specificity of a given microorganism was its ability to reproduce the characteristic lesions in certain monkeys known to be susceptible to trachoma. Irrespective of morphological and other properties, every kind of microorganism isolated from the trachomatous materials was tested on

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[31] Noguchi, H., Muller, H. R., Torres, O., Silva, F., Martins, H., Ribeiro dos Santos, A., Vianna, G., and Biao, M., Experimental studies of yellow fever in northern Brazil, Monograph of The Rockefeller Institute for Medical Research, No. 20, New York, 1924.

[32] Avery, O. T., *J. Am. Med. Assn.*, 1918, lxxi, 2050.

[33] Noguchi, H., *J. Exp. Med.*, 1912, xvi, 199, 211.

more than one monkey. When several strains of the same microorganism were isolated, they were combined and inoculated into two or more monkeys; in case of positive results, each of the group of strains was subsequently tested separately.

Materials Investigated.—Advanced untreated cases of trachoma are unusual in a country like the United States, where the disease is not endemic. They occur, however, among the students admitted to the government schools for Indians, and through the kind intervention and personal assistance of Dr. F. I. Proctor, in cooperation with the Department of the Interior, arrangements were made for me to be present at the routine examination of the students of the Albuquerque Indian School and select suitable cases for study.

The diagnoses were made by Dr. Parlett, the school physician, and Dr. P. Richards, of the U. S. Indian Field Service, stationed in Fort Defiance, Arizona, who came to Albuquerque expressly to cooperate in the surgical procedures. These procedures were only such as are ordinarily employed in the treatment of the disease; they would have been resorted to irrespective of my investigation.

On May 11 and 12, 1926, eleven cases were selected and the removed tissues utilized for the inoculation of animals and culture media. Film preparations were immediately made and several kinds of plate media inoculated. A portion of the tissue was ground with sterile saline in a sterile mortar and the suspension used for the inoculation of various media in tubes, as described further on. Part of the suspension from each case was set aside for the purpose of inoculating monkeys. In one instance (Case 5) a small portion of the diseased tarsus of the left upper lid was utilized later for the inoculation of a chimpanzee and an ourang-utan, as well as for histological study.

Because of the large amount of experimental work which proved necessary in the cultural analysis of each case, it was possible to utilize the material from only the first five of the eleven cases. Other materials furnished by Dr. Eilers, of the U. S. Indian Medical Service Hospital in Albuquerque, on May 12, had to be set aside, for the same reason.

The clinical histories of Cases 1 to 5 are recorded below. In all instances the disease had been present for 2 years or longer, and scar tissue had already formed, ruling out the possibility of confusion with follicular conjunctivitis. With the exception of Case 1, the pupils were all reexamined by Dr. Richards in May, 1927, one year after my visit.

Case 1, B. B.; this student came from another school. Duration of disease not accurately known, but scar tissue was already present.

Case 2, L. A., Pueblo, 15 years old, stated that he had had sore eyes for more than 3 years. Reexamination in May, 1927, showed that he still had some active trachoma, with scar tissue formation.

Case 3, J. F., Pueblo, 12 years old, stated that she had had trachoma about 3 years. Reexamination in May, 1927, showed scar tissue formation in the upper fornices but not much evidence of active trachoma.

Case 4, D. P., Pueblo, 9 years old, thought her eyes had been sore for about 2 years. Reexamination in May, 1927, showed trachoma still active, with considerable scar tissue formation.

Case 5, L. P., Pueblo, 12 years old, stated that he had had trachoma for at least 2 years. Reexamination in May, 1927, disclosed trachoma still active, with scar tissue formation.

Pathological Findings.—Trachoma as it occurs among the American Indians is the same as the so called classical trachoma encountered elsewhere. During my brief stay in Albuquerque I was given opportunity to observe the disease in its various stages (Plate 1). At the government school I saw many well marked but early cases (Figs. 1–2), while at the hospital of the U. S. Indian Medical Service Dr. Eilers showed me many advanced cases with scarred conjunctivæ, entropionized lids, trichiasis, and varying degrees of pannus (Figs. 3–4).

Histological examination of the excised conjunctiva from Albuquerque Case 5 revealed diffuse lymphocytic infiltration and follicles occupying the subepithelial layer, and the flattening of the epithelial cells over the follicle; there was but little tendency to cicatrization in this case. Later a number of excised tarsi from cases occurring in Fort Defiance, Arizona, and in Rice, Arizona, forwarded to me through the kindness of Dr. P. Richards and Dr. J. S. Perkins, respectively, were also studied (Plates 3–6). These all show the typical trachomatous lesions in different stages, with considerable increase of newly formed connective tissues in the conjunctival mucosa and submucosa.

The diameter of the follicles varies from 0.5 to 2 mm. or more. Their structure is quite characteristic. At the periphery are layers of lymphocytes. Some of the large follicles show a central area filled with large mononuclear cells, while in others this formation is obliterated by an irregular intermixing of small lymphocytes and large mononuclears; occasionally cells from the proliferating epithelium intrude into the follicles. Leber's cells and plasma cells occur in varying number. Mast cells are quite numerous along the interstitial spaces, sometimes even within the

follicles. The papillæ are usually crowded with lymphocytes, and the epithelium is unevenly thickened by cellular proliferation. Goblet cells may become enclosed between the papillæ, giving the appearance of a mucous gland (the so called mucous gland of Iwanoff). Polynuclear leucocytes are present in small numbers, but no giant cells occur. The lymphocytic infiltration and follicles are more abundant in the fornices and along the upper border of the superior tarsus. It is known that at the florid stage these changes become so general as to involve the entire tarsal conjunctiva. In tarsi removed at the beginning of cicatrization, the follicles contain fine connective tissue fibrils which are contiguous with the connective fibers that surround the follicles. Small blood vessel capillaries are sometimes seen. The adenoid layer is more fibrous at this stage, and the papillæ also are pervaded by the newly formed fibrils and fibroblasts. The epithelial layer is thickened except where a rupturing follicle has pushed the flattened cells out into a single row or has broken through them. In some cases rows of infiltrated and hyperplastic papillæ over the tarsus present a striking picture. In the more advanced stages, when the degree of fibrosis is much greater, there is a diminution in the number and size of follicles and infiltrating lymphocytes.

Sections showing the normal histology of the human conjunctiva (Plate 2) for comparison were kindly furnished by Drs. M. Cohen and A. Knapp, of New York, and Dr. C. E. Schradieck, of Providence, Rhode Island.

I may mention here that the inclusion bodies of Halberstädter and Prowazek could not be demonstrated in any of the Albuquerque cases.

Cultivation Experiments.

In the cultivations conducted by us the special media employed were:

1. Blood agar plates, to which had been added a mixture of dextrose, maltose, saccharose, galactose, inulin, and dextrin to give a final concentration of 1 per cent of each carbohydrate. The sugar mixture was filtered through a Berkefeld filter N, and thus sterilization by heat was avoided. Defibrinated blood (horse or rabbit) was used in a final concentration of 20 per cent of the medium. The agar employed was the hormone broth agar of Huntoon.
2. Avery's hemoglobin sodium oleate agar plates, as used for the cultivation of the influenza bacillus group.
3. The semisolid leptospira medium, as employed for the cultivation of such highly parasitic organisms as leptospiras, *Bartonella bacilliformis*, and flagellates, which grow only slightly or not at all on other media. It consists of 8 parts of 0.9 per cent sodium chloride solution, 1 part of fresh rabbit serum, 1 part of 2 per cent nutrient agar, and about 0.1 part of laked rabbit erythrocytes.

TABLE I.

Representative Findings in Plate Cultures.

	Colonies	Morphology	Gram	Subcultures					
				30°C.			37°C.		
				Leptospira medium	Blood slants	Plain slants	Leptospira medium	Blood slants	Plain slants
				Case 1.					
1	Round, slightly raised, bluish gray colony	Minute bacilli	−	Grayish	Grayish, shiny, smooth, minute colonies	No growth	Grayish, poor growth	No growth	No growth
2	Similar	" "	±	"	Similar	" "	Grayish, poor growth	" "	" "
3	Light lemon, moist, viscid	" "	−	Faintly lemon	Light lemon, moist, mucoid	Light lemon, mucoid	Light lemon	Poor "	" "
4	Pinkish, shiny, raised	Markedly pleomorphic bacilli	−	Light pink	Heavy moist colonies	Reddish orange	Pinkish heavy scum	Heavy "	Heavy reddish growth
5	Yellowish, moist, raised	Short xeroid	±	Yellowish	Yellowish, moist, raised	Salmon, moist	Yellowish	Yellowish	Yellowish
6	Dry, grayish	Ordinary "	+	Grayish, granular	Dry, grayish	Dry, grayish	Grayish, granular	Grayish, dry	Grayish, dry
7	Light lemon, moist, viscid	Sarcinoid	±	Light yellow	Light yellow, moist, viscid	Light lemon, moist, mucoid	Light brown, sticky	Poor growth	No growth
8	Grayish, moist	"	−	Grayish	Grayish, moist, viscid	No growth	Grayish	" "	" "
9	" "	Staphyloid	+	"	Grayish, moist	Whitish gray	"	Dark grayish	Grayish white

Case 2.

1	Grayish, dry colony	Ordinary xeroid	+	Grayish granular	Grayish, dry colonies	Dry, grayish	Grayish, granular	Grayish, dry colonies	Grayish, dry colonies
2	Light pinkish, raised, moist	Short xeroid	+	Light pink	Heavy pinkish growth	Salmon, moist heavy growth	Pinkish scum	Heavy pinkish growth	Heavy pinkish moist colonies
3	Yellowish brown, moist, viscid	Sarcinoid, medium size	—	" yellow	Yellowish shiny growth, mucoid	Light yellow, mucoid	Light brown	Poor growth	No growth
4	Large, moist, grayish, viscid	Sarcinoid, large	—	Grayish	Grayish	Grayish, moist, viscid	Grayish	Grayish, moist, mucoid	" "
5	Whitish	Staphyloid	+	"	Whitish	Whitish, moist	Whitish gray	Whitish	Whitish gray

Case 3.

1	Semitransparent, bluish gray, moist	Minute bacilli	—	Grayish	Grayish, shiny, small bluish gray colonies	No growth	Grayish, poor growth	No growth	No growth
2	Light lemon, mucoid	" "	—	"	Light lemon, mucoid	Light lemon, moist, mucoid	Light yellow	Light lemon, mucoid	" "
3	Mixed colony	" "	—	" scum	Grayish	Heavy yellow, mucoid	Grayish	Grayish	Grayish
		Coarse "	+						
4	Grayish, moist	Xeroid	+	" heavy	" moist	Salmon, moist	"	" moist	" moist
5	Dry, grayish	"	+	" granular	Dry, grayish colonies	Dry, grayish	Dry, grayish colonies	" granular	" dry colonies
6	Whitish	Staphyloid	+	Grayish	Whitish	White, moist	Whitish	Whitish	Whitish gray

TABLE I—*Concluded.*

	Colonies	Morphology	Gram	Subcultures					
				30°C.			37°C.		
				Leptospira medium	Blood slants	Plain slants	Leptospira medium	Blood slants	Plain slants
				Case 4.					
1	Small, round, moist, grayish, viscid	Minute bacilli	–	Grayish	Grayish, moist, viscid	No growth	Grayish, poor growth	—	No growth
2	Similar	" "	–	"	Grayish, moist, mucoid	" "	Grayish	Grayish, moist, viscid	" "
3	Lemon, moist, viscid	" "	–	Light lemon	Light lemon, viscid	Light lemon, mucoid	Light lemon	Lemon, viscid	" "
4	Mixed colony	Xeroid	+	Yellowish scum	Yellowish	Thick, creamy mucoid	Yellowish	Yellowish	Yellowish
		Sarcinoid	–						
5	" "	Xeroid	+	Grayish	Brownish gray	Light greenish	Brownish gray	Brownish gray, viscid	Brownish, viscid
		Sarcinoid	–						
6	" "	Xeroid	+	Greenish	Whitish	Dry, grayish and white colonies	Whitish	Whitish	Whitish
		Staphyloid	+	Gray					
7	Dry, grayish	Xeroid	+	Grayish, granular	Dry, grayish	Yellowish, dry, granular	Grayish, granular	Grayish, dry	Dry, grayish
8	Whitish	Staphyloid	+	Grayish	Whitish	White, moist	Whitish	Whitish	Whitish
9	"	"	+	"	"	" "	"	"	"

Case 5.

1	Grayish, moist, viscid	Minute bacilli	—	Grayish	Grayish, moist, viscid	No growth	Grayish	No growth	No growth
2	Grayish, moist, viscid	“ “	—	“	Grayish, shiny, viscid	“ “	“	“ “	“ “
3	Lemon, raised, viscid	“ “	—	Light lemon	Lemon, raised, mucoid	Light lemon, mucoid	Light lemon	Lemon, moist, mucoid	“ “
4	Light yellow, viscid	“ “	±	“ “	Yellowish, mucoid	Light lemon, mucoid	Yellowish	Yellowish brown, mucoid	Yellowish brown, mucoid
5	Brownish, moist, viscid	Sarcinoid	—	Brownish	Brownish, raised, viscid	Brownish, mucoid	Brownish	Brownish, shiny, mucoid	No growth
6	Similar	“	—	“	Similar	Light rose	“	Poor growth	“ “
7	“	“	±	“	“	“ “	“	No “	“ “
8	Grayish, shiny, viscid	“ large	—	Grayish	Grayish, viscid	Grayish, mucoid	Grayish	Grayish, mucoid	Grayish, mucoid
9	Brownish, moist, viscid	“	—	Brownish	Brownish, mucoid	Brownish, mucoid	Brownish	Brownish, mucoid	No growth
10	Similar	“	—	“	Similar	Brownish, mucoid	Similar	No growth	“ “
11	Dry, grayish	Xeroid	+	Light brown	Dry, grayish	Salmon, viscid	Grayish, granular	Dry, grayish	Dry, grayish
12	Whitish	Staphyloid	+	Grayish	Whitish	White, moist	Whitish	Whitish	Whitish

4. Ascitic agar with fresh rabbit kidney, as used for cultivating such anaerobic microorganisms as treponemata.

The materials expressed from the follicles with sterile ring forceps were directly smeared over the surface of the plate media. In the case of the leptospira medium, which was contained in tall test-tubes, a saline suspension of pieces of tissue thoroughly ground in a sterile mortar was inoculated in ascending dilutions among a single or sometimes double series of six tubes, with the hope of obtaining pure cultures in the higher dilutions. The tubes of ascitic agar medium containing tissue were inoculated by means of capillary pipettes, the liquid being forced into the solid medium by means of a syringe.

The plates were sealed with adhesive tape, both before and after inoculation, to prevent drying of the medium. A part of the plates and tubes was kept at room temperature (15–20°C.) for 6 days and then placed at 30°C., while several duplicate sets were incubated at 37°C. for the first 48 hours and then placed at room temperature. 9 days after inoculation, isolation of the cultures was begun, and it was continued at intervals for at least 4 weeks.

Blood Agar Plates.—Every colony was examined in fresh preparations by dark-field illumination and in films stained by Gram's method. Transfers were made to leptospira medium, blood agar slants, and plain agar slants half filled with broth, one set of subcultures being kept at 30°C. and another at 37°.

The blood agar plates showed few colonies, which were usually well separated. The results of the examination are given in Table I.

The microorganisms isolated from the original blood agar plates (71 cultures in all) fall into the following five groups:

1. Staphylococcus, obtained in all cases.
2. *Corynebacterium xerosis*, obtained in all cases.
3. Sarcinoids, obtained in Cases 1, 2, 4, and 5.
4. Minute, motile, Gram-negative bacillus, producing yellowish pigment and mucin, growing better at 30°C. than at 37°, obtained from Cases 1, 3, 4, and 5.
5. Minute, Gram-negative bacillus resembling *Bacillus xerosis*, but smaller, non-chromogenous, the growth somewhat viscid. It was first thought to be non-motile but was found later to be motile under certain cultural conditions. It grows on leptospira medium at 30°C. as well as 37°. On blood agar slants or plates it grows at 30° but only slightly or not at all at 37°. It does not grow on plain agar or broth. This type was isolated from Cases 1, 3, 4, and 5.

Leptospira Medium.—In the leptospira medium, in 14 × 200 mm. tubes, inoculations consisted of ascending tenfold dilutions 1:10 to 1:100,000 of the original suspensions. Usually a mixed growth was obtained. The results are summarized in Table II.

TABLE II.

Cultures on Leptospira Medium.

1. Mixed Suspension of Follicle Contents of Cases 1 and 2.		
Undiluted suspension		Staphylococcus, *C. xerosis*, sarcinoids
1:10	(1 tube)	The same
1:100	(" ")	" "
1:1,000	(" ")	" "
1:10,000	(" ")	" "
1:100,000	(" ")	" " and also a minute *Gram-negative bacillus*
2. Suspension of Follicle Contents of Case 3.		
Undiluted	(1 tube)	Staphyloids, sarcinoids, *C. xerosis*, and minute *Gram-negative bacilli*
1:10	(2 tubes)	The same
1:100	(" ")	No growth in one tube; one sarcinoid in the other tube
1:1,000	(" ")	No growth
1:10,000	(" ")	Staphyloids, sarcinoids, *C. xerosis*, minute *Gram-negative bacilli*
1:100,000	(" ")	No growth.
3. Suspension of Follicle Contents of Case 4.		
Undiluted	(1 tube)	Sarcinoids, xeroids, staphyloids, *Gram-negative bacilli*
1:10	(2 tubes)	The same
1:100	(" ")	" "
1:1,000	(" ")	No growth
1:10,000	(" ")	Pure growth of *minute Gram-negative bacilli* in one tube, no growth in the other
1:100,000	(" ")	No growth
4. Suspension of Follicle Contents of Case 5.		
Undiluted	(1 tube)	Staphyloids, sarcinoids, *C. xerosis*
1:10	(2 tubes)	The same
1:100	(" ")	" " also *minute Gram-negative bacilli*
1:1,000	(" ")	*Minute Gram-negative bacilli* and few sarcinoids
1:10,000	(" ")	Same as 1:1,000
1:100,000	(" ")	No growth

The organisms growing on the leptospira medium were identical with those which developed on the blood agar plates. The minute Gram-negative bacilli above described were present in all cases, and *in the tube inoculated with the 1:10,000 dilution of the suspension from Case 4 they were obtained in pure culture.* By repeated plating on freshly prepared blood agar plates, kept at 30°C. and at 37°, both varieties of Gram-negative bacilli, corresponding to those which had been obtained on plates, were isolated from the mixed cultures.

Hence it follows that two varieties of Gram-negative bacilli were isolated in the blood plates and leptospira medium cultures. Later studies proved these varieties of bacilli to be serologically distinct.

Avery Plates.—The colonies which developed on the Avery plates were less numerous than those on the blood agar plates. The material from Case 1 gave rise to several brownish and a few whitish gray colonies of Gram-positive coccoids. Case 2 yielded several colonies of staphylococci and of *C. xerosis.* Case 3 yielded staphylococci only. The plates from Cases 4 and 5 contained staphylococci, *C. xerosis,* and sarcinoids.

Tissue-Ascites-Agar Medium.—This medium, which was in tubes 14 × 200 mm., was examined 26 days after inoculation. Staphylococci, sarcinoids, and *C. xerosis* were the only organisms which developed.

Morphological and Cultural Properties of the Gram-Negative Bacilli.

The morphology and growth characteristics of the Gram-positive cocci and bacilli, as well as the large spherical bodies, often Gram-negative, left little doubt that they were the organisms known as staphylococci, *Bacillus xerosis,* and sarcinæ. The two kinds of minute Gram-negative microorganisms isolated, however, have not so far been identified with any organism previously described, and attention should be concentrated on them.

One of these bacilli measures 0.25 to 3 μ by 0.8 to 1.4 μ, is actively motile under all cultural conditions, and retains its motility for many days or even weeks. It grows well on plain agar, producing light lemon or yellowish pigments and an abundance of mucin, especially in and near the condensation water. In broth it grows diffusely and forms within several days slight pigment and a considerable amount of mucin. It is non-sporogenous and apparently is devoid of a capsule.

It does not give a putrefactive odor, but ferments several sugars, and multiplication is somewhat better at 30°C. than at 37°. This bacillus arrested our attention in the beginning, because it appeared unusual, but it proved to be without pathogenic action when tested on the monkey conjunctiva.

The other Gram-negative bacillus bears a morphological resemblance to *B. xerosis,* though it is smaller and less resistant to decolorization. For the sake of brevity it was entered on the records as "minute Gram-negative *xerosis.*" In older surface cultures all kinds of involuted forms appear: club shapes, polygonal, oval, or even barred, curved, and short bifid forms. But for my resolution to test every organism for pathogenic action, this bacillus would have been disregarded as a variant of the harmless *B. xerosis.* As a matter of fact, the inoculation of this organism in pure culture into the conjunctivæ of monkeys produces a form of granular conjunctivitis closely resembling trachoma in man.

Close study of this organism revealed certain differences from *B. xerosis,* such as inability to grow on plain agar or broth at any temperature (15–37°C.). Strains of the bacillus which have been repeatedly subcultured for the past 10 months grow well on plates or slants containing defibrinated or citrated blood, citrated plasma, or serum of the horse or rabbit, at 15–30°C., but only slightly or often not at all at 37°C. (Table III, Plates 8–9). The growth on horse blood agar is much more vigorous than on rabbit blood agar. On both the semisolid and fluid leptospira media the bacillus grows well at 37°C. as well as at lower temperatures—15–30°C. (Plate 7).

The colonies on blood agar plates incubated at 30°C. appear in about 48 hours as minute shiny, somewhat raised, almost transparent or slightly grayish round points which, in the following days, gain in size and acquire a grayish opalescence and are of somewhat sticky consistence (Plate 8). Old colonies have a faint brownish or yellowish tint. The morphological appearances of the organism when grown on various media at 30°C. or 37°C. are shown in Plates 10–13.

In the semisolid leptospira medium, the growth is in the form of a diffuse, grayish white, delicate zone, 1 cm. deep, which may follow the stab downwards (Fig. 21). The liquid leptospira medium is diffusely but slightly clouded by the growth; in old cultures a sticky grayish sediment is found at the bottom of the tube, but no surface scum or pellicle is formed. In this latter respect the bacillus resembles *Bacterium monocytogenes,*[34] which organism, however, grows well on ordinary agar slants and in broth.

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[34] Murray, E. G. D., Webb, R. A., and Swann, M. B. R., *J. Path. and Bact.,* 1926, xxix, 407. Pirie, J. H. H., *Pub. South African Inst. Med. Research,* 1927, iii, 185. I am indebted for cultures of this microorganism to Dr. J. C. G. Ledingham, Director of the National Collection of Type Cultures at the Lister Institute.

TABLE III.

Trachoma—Albuquerque Strain 1. 11 Months after Isolation.
Growth on Various Culture Media.

May 17, 1927.

Freshly prepared media	Days of incubation	Rabbit				Horse			
		30°		37°		30°		37°	
		Plate	Slant	Plate	Slant	Plate	Slant	Plate	Slant
Blood agar (15%)	3	<+	<+	−	−	<+	<+	−	−
	4	<+	<+	−	−	+	+	<+	−
	7	+	+	−	−	++	++	+	−
Same + sugars (1%)	3	<+	<+	<+	−	+	+	+	−
	4	+++	+++	+	−	++++	+++	++	++
	7	+++	+++	++	−	++++	++++	+++	+++
Serum agar (10%)	3	−	−	<+	−	−	±	<+	−
	4	<+	++	+	−	<+	++	++	−
	7	+	++	+	−	<+	++	++	−
Same + laked blood (2%)	3	+	<+	<+	−	+	+	+	−
	4	++	++	+	−	++	++	++	−
	7	+++	+++	+	−	+++	+++	++	−
Citrated plasma agar (20%)	3	−	−	<<+	−	−	−	−	−
	4	±	+	+	−	±	<+	−	−
	7	<<+	+	+	−	<+	+	<<+	−
Same + laked blood (2%)	3	−	−	<<+	−	−	−	−	−
	4	<<+	<<+	+	−	+	++	++	−
	7	+	+	+	−	+	++	++	−
Nutrient agar	3	−	−	−	−	−	−	−	−
	4	−	−	−	−	−	−	−	−
	7	−	−	−	−	−	−	−	−
Same + laked blood (2%)	3	−	−	−	−	−	−	−	−
	4	<<+	<<+	+	−	+	+	+	−
	7	<+	<+	+	−	+	+	+	−
Leptospira medium	3	<+		+					
	4	+++		+++					
	7	++++		++++					

One of the striking features of the bacillus is its motility. The motility was overlooked by me until April, 1927, nearly 11 months after the isolation of the first strain, the reason being that I maintained all strains in leptospira medium because of the absence or uncertainty of growth on any other media tried—among them Loeffler's coagulated serum slants, Francis's cystine slants, Petroff's egg slants, glycerol agar slants, and even blood agar slants. On the leptospira media, both semisolid and fluid, incubated either at 30° or at 37°, the organism is non-motile. Surface colonies also on blood agar plates are usually non-motile. However, on one occasion, while examining the condensation water of a horse blood agar slant, incubated for 38 hours at 30°C., I noticed many actively motile forms. At first I feared that contamination had taken place, but on further study I found that a non-motile culture grown on the leptospira medium develops into a motile one when inoculated on horse blood agar slants and incubated at 30°C. The motility is readily demonstrated by examining the condensation water under the dark-field microscope. Still later I noticed that colonies developing on the blood agar surface or on the leptospira media kept at a temperature of 15°C. contain many actively motile forms, while those kept at 30° or 37° lack motility.

The bacillus moves by means of a single flagellum, which arises usually from one of the poles, but occasionally appears as if attached to one side (Plate 10, Figs. 41–42). The finding of motility, and the type of flagellum, together with the other properties of this microorganism, definitely remove it from the great group of corynebacteria to which diphtheroids of all descriptions and sources belong.

Individuals in a young culture measure 0.25 to 0.3μ by 0.8 to 1.2μ. In old cultures on a blood agar surface involution forms of larger dimension and irregular shapes predominate. Growth occurs at hydrogen ion concentrations ranging from pH 6.8 to 8.8, the optimum being in the neighborhood of pH 7.8. The organism is non-sporogenous and bile-resistant. Under strictly anaerobic conditions no growth occurred. In some respects (motility at low temperature, polar flagellum, optimum growth on leptospira medium) the organism recalls *Bartonella bacilliformis*. When freshly isolated it is apparently without appreciable action on carbohydrates, but tests made repeatedly for 6 months or longer after the isolation show that in the presence of many carbohydrates it slowly acidifies the medium, though without inducing coagulation. No gas is produced.

In Table IV are given the results of fermentation tests on 20 different carbohydrates contained in a fluid medium (Hiss serum water) to which was added 10 per cent horse serum and 0.2 per cent horse hemoglobin solution. The cultures were kept at 30°C. and the results read at different periods. For the purpose of comparison 2 strains of *Bacterium monocytogenes*,[34] a strain of *C. xerosis*, 3 strains of *C. diphtheriæ*,[35] 1 strain of *C. pseudotuberculosis ovis*,[35] and 1 strain of *C. hofmanni*[35] were added to the series. It was found that the strains of *mono-*

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[35] Obtained from the American Type Culture Collection.

TABLE IV.

Fermentation Tests.

	Albuquerque strains					Known organisms for comparison							
	Case 1	Case 3	Case 4	Case 5	Chimpanzee "Kitty"	*B. monocytogenes* Harvey	*B. monocytogenes* Murray	*C. xerosis* "Kitty"	*C. diphtheriæ* 170	*C. diphtheriæ* 295	*C. diphtheriæ* 325	*C. pseudotuberculosis ovis*	*C. hofmanni*
1. Glucose	++	++	++	++	++	++++	++++	++++	++++	++++	++++	<+	−
2. Levulose	++	++	++	++	++	++++	++++	++++	++++	++++	++++	<+	−
3. Mannose	++	++	++	++	++	++++	++++	++++	++++	++++	++++	<+	−
4. Saccharose	++	++	++	++	++	−	−	++++	−	−	−	−	−
5. Raffinose	+	+	+	+	++	−	−	−	−	−	−	−	−
6. Inulin	+	+	+	+	+	−	−	−	−	−	−	−	−
7. Galactose	++	++	++	++	++	−	−	−	+++	++	++++	−	−
8. Maltose	++	++	++	++	++	+++	+	+	−	−	−	−	−
9. Salicin	++	++	++	++	++	++++	++++	+	−	−	−	−	−
10. Xylose	++	++	++	++	++	+	+	+	−	−	−	<+	−
11. Mannitol	++	++	++	++	++	−	−	−	−	−	−	−	−
12. Dextrin	++	++	++	++	++	++++	++	−	++	++	++	−	−
13. Arabinose	++	++	++	++	++	+	+	−	−	−	−	<+	−
14. Amygdalin	++	++	++	++	+	++++	++++	−	−	−	−	−	−
15. Lactose	++	++	++	++	++	−	−	−	−	−	−	−	−
16. Dulcitol	−	−	−	−	−	−	−	−	−	−	−	−	−
17. Rhamnose	+	+	+	+	+	++	++	−	−	−	−	−	−
18. Trehalose	<<+	±	±	<<+	±	−	−	+	−	−	−	−	−
19. Sorbitol	−	−	−	−	−	−	−	+	−	−	−	−	−
20. Inositol	−	−	−	−	−	−	−	−	−	−	−	−	−
21. None	−	−	−	−	−	−	−	−	−	−	−	−	−

Readings after 14 and 21 days at 30°C.

cytogenes, *diphtheriæ*, and *xerosis* all attacked glucose, levulose, and mannose so actively that the media became coagulated in about 10 days. The *monocytogenes* also coagulated salicin and amygdalin with about the same rapidity, while the *diphtheriæ* attacked galactose also. On the other hand, 5 strains of the present organism (4 from Albuquerque cases and 1 isolated from the chimpanzee, "Kitty," inoculated with this organism) failed to coagulate any of the media notwithstanding definite acidification in most instances.

The bacillus described appears to be entirely devoid of pathogenic action for rabbits, guinea pigs, rats, and mice. The absence of effect in rabbits is in striking contrast to the fatal action of *B. monocytogenes*.

I have gone into detail in describing the properties of this bacillus because, as will be shown later on, it alone, of the various strains isolated, produced a granular conjunctivitis in monkeys and apes. For this reason the name *Bacterium granulosis* is proposed for it.

Direct Inoculation of Animals with the Trachomatous Materials.

Four young *Macacus rhesus* monkeys were taken to Albuquerque for the purpose of making direct patient to animal inoculations. On May 11, 1926, parts of the saline suspensions of trachomatous tissues from the five cases employed for cultures were pooled and the mixture injected[36] with a tuberculin syringe into the everted subconjunctival tissue in the upper left palpebral region near the upper border of the tarsal plate. The 0.3 cc. injected was sufficient to cause mechanical bulging of the conjunctiva. At the same time the surface of the tarsal conjunctiva was scratched lightly and punctured with the point of the needle. The right eye served as control.

The four monkeys were taken back to The Rockefeller Institute and kept under observation for about 8 months. Except for a slight traumatic irritation lasting a few days, the conjunctivæ remained permanently normal; the inoculation must be regarded as negative.

On May 20 a chimpanzee and an ourang-utan were inoculated with the material excised from Case 5 at Albuquerque and kept 9 days at a refrigerator temperature during the return journey to New York. The material was kept moist and in a sterile sealed tube, and was ground into a fine condition and suspended in saline immediately

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[36] The injections were made under local anesthesia (novocaine). During removal of tissues ether anesthesia was employed.

previous to the injection into the right subconjunctival tissue in the manner just described. Except for a slight traumatic irritation lasting a few days, no other pathological condition developed during the 8 months of observation.

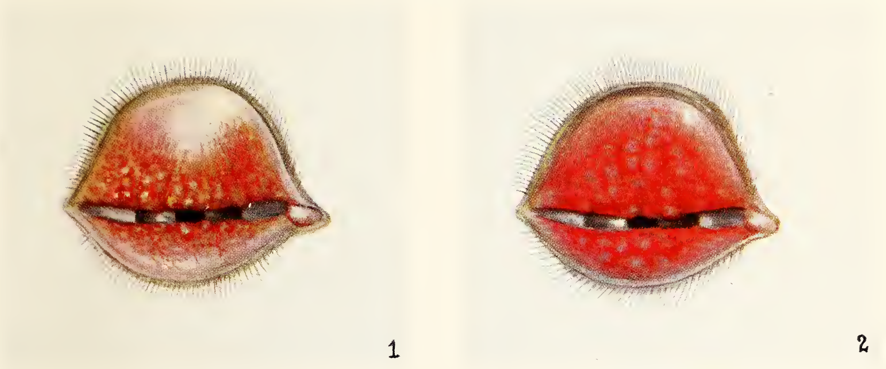
SUMMARY.

According to clinical and pathological studies, the granular or follicular conjunctivitis prevailing among the American Indians at a reservation in New Mexico is to be classed with trachoma as observed in other parts of the world.

Through the employment of particular kinds of culture media for inoculation with materials obtained from trachomatous eyes, and by the use of low temperature incubation, not only have a number of common bacterial forms—cocci, bacilli, and sarcinæ—been secured, but in addition two distinct varieties of peculiar Gram-negative motile bacilli, from four of the five cases of trachoma studied bacteriologically. The two kinds of motile bacilli are readily distinguished, since one grows well at 37°C., as well as at lower temperatures, on ordinary culture media, while the other requires media containing animal blood and thrives best at temperatures from 15–30°C. The latter organism does not multiply when oxygen is completely excluded. It bears resemblance to *Bacterium monocytogenes* but differs from the latter in its inability to grow on ordinary media at 37°C., in its non-pathogenicity for rabbits, and in serological reactions. Under conditions of multiplication considered unfavorable, the bacilli assume forms found in the group of corynebacteria, but differ from these in showing motility under particular growth conditions. Single polar flagella are carried by the bacilli, which do not seem to have been described before. These bacilli take on a special significance in virtue of the fact, to be described in a later part of this report, that they are able to induce granular conjunctival inflammation on inoculation into monkeys. Because of this property the name *Bacterium granulosis* is being proposed for the organism.

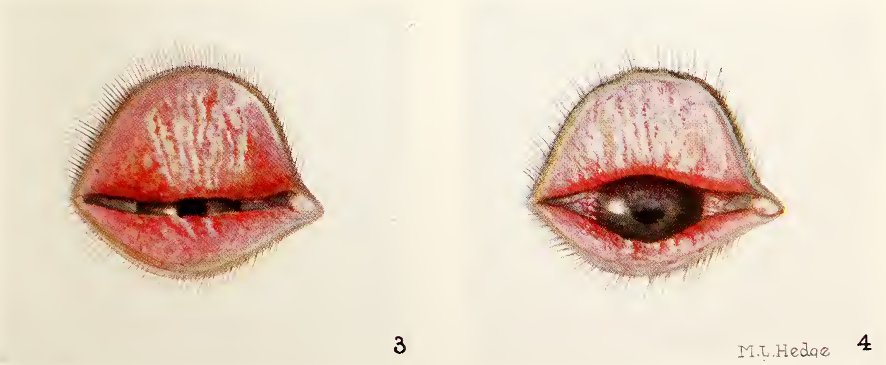
Unsuccessful attempts were made to induce trachoma in *rhesus* monkeys, a chimpanzee, and an ourang-utan, by direct inoculation of materials taken from the cases of Indian trachoma described in this paper.

Trachoma.



Early stage.

Florid stage.

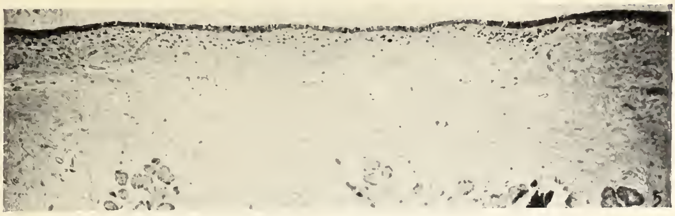


Beginning of cicatrization.

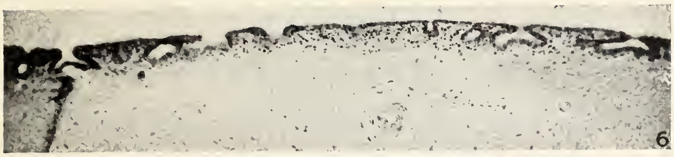
Cicatrization with pannus.

(Noguchi: Etiology of trachoma.)

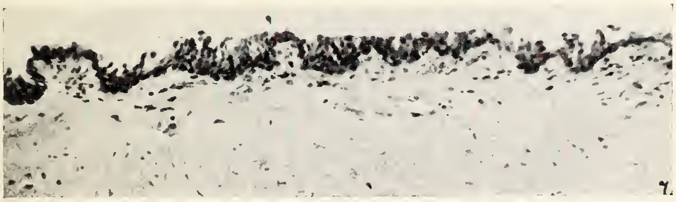
Human conjunctiva, practically normal. Giemsa's stain.



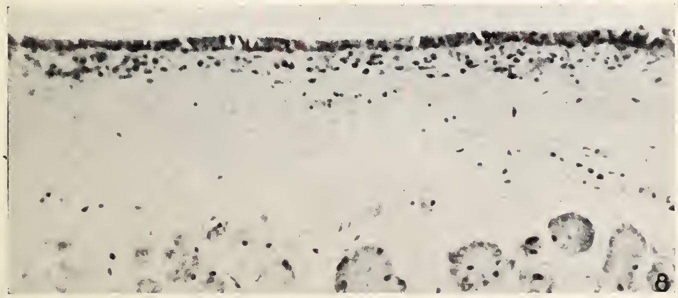
Tarsal conjunctiva, middle portion. × 52.



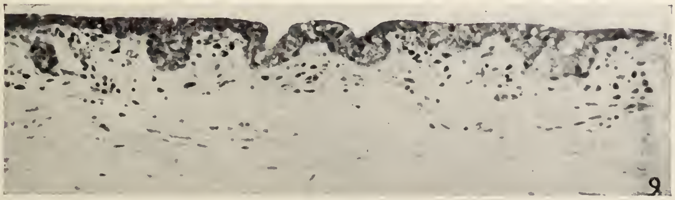
Same, towards the free edge of the lid. × 52.



Same, towards the fornix. × 156.



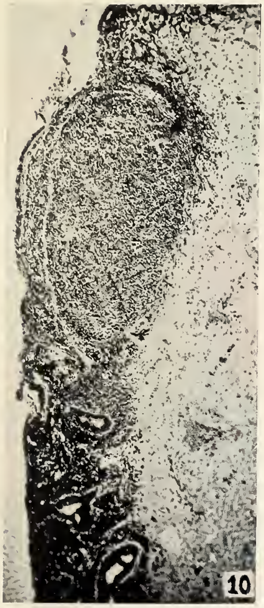
Same, middle portion. × 156.



Same, towards the free edge. × 156.

(Noguchi: Etiology of trachoma.)

Trachomatous conjunctivæ. Giemsa's stain. × 52.



Case E. P.

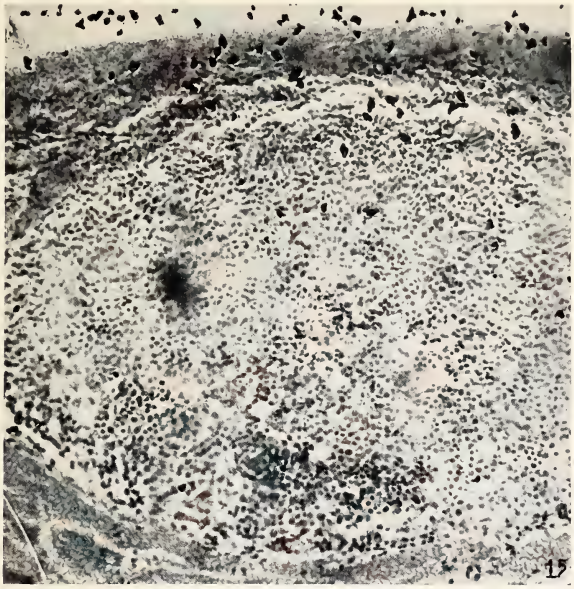
Case E. F.

Case A. G.

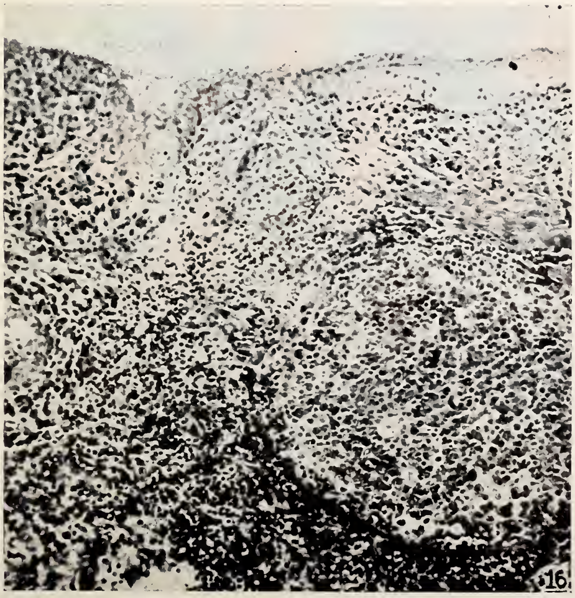
Case R. M.

Fort Defiance Case 1.

Trachomatous conjunctivæ. Giemsa's stain. × 156.



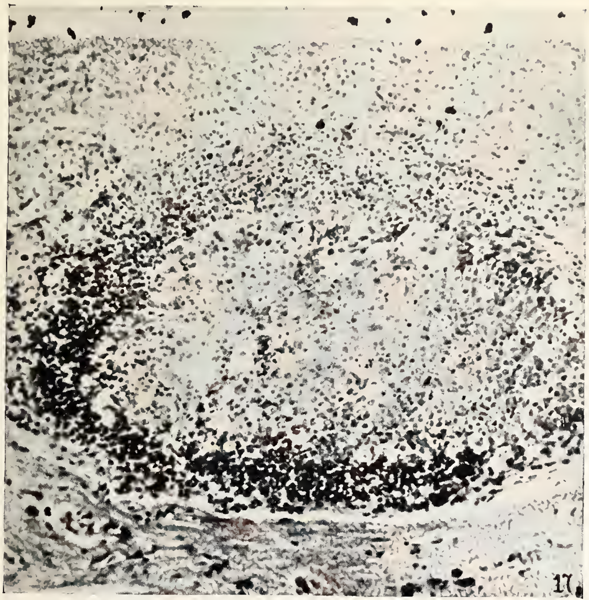
A portion of a large follicle. Fort Defiance Case 1.



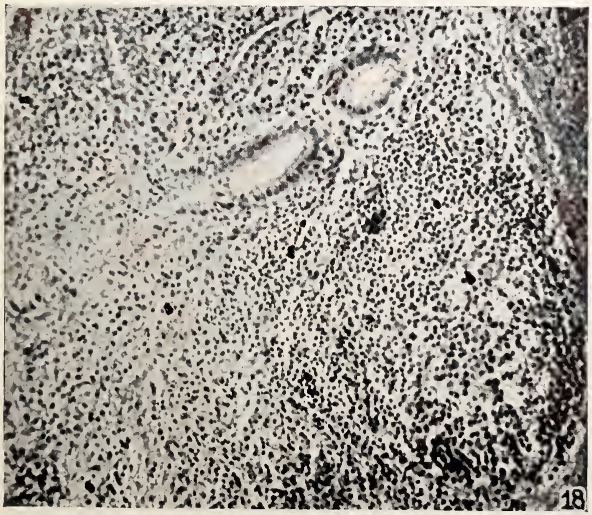
A follicle of medium size, with a diffuse perifollicular infiltration. Fort Defiance Case 1.

(Noguchi: Etiology of trachoma.)

Trachomatous conjunctivæ. Giemsa's stain. × 156.

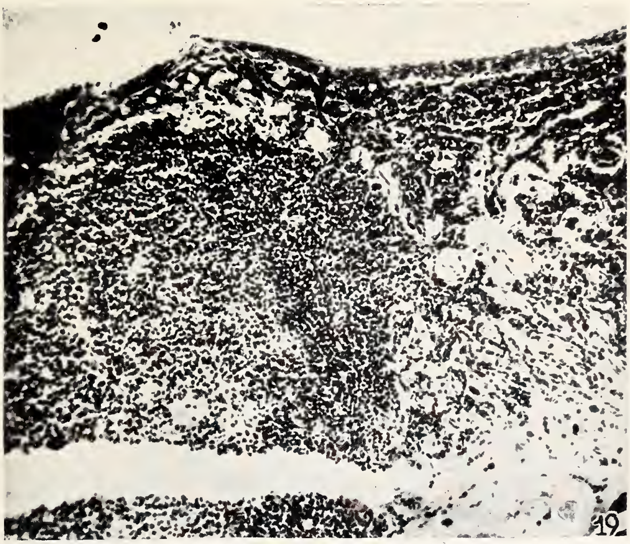


A follicle with ill staining center surrounded by a zone of small, deeply staining monocytes. Fort Defiance Case 2.

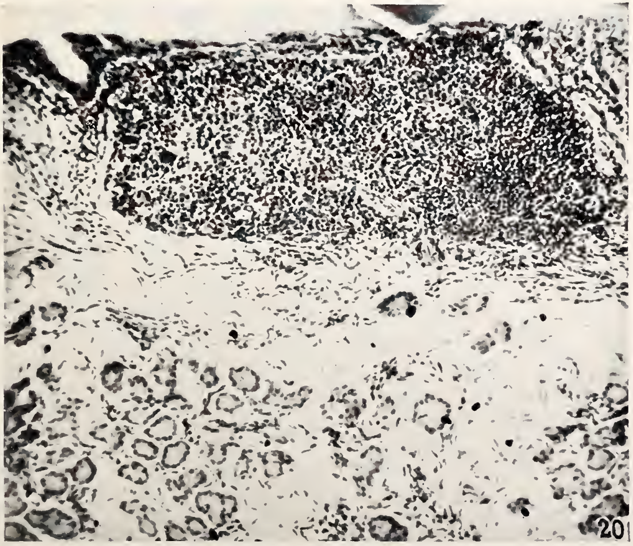


An ill defined follicle contiguous with the infiltrated subepithelial area. Fort Defiance Case 2.

Trachomatous conjunctivæ. Giemsa's stain. × 156.



A densely packed follicle showing predominance of small round cells. Case E. F.



A similar follicle situated immediately beneath the epithelium. Case A. G.

(Noguchi: Etiology of trachoma.)

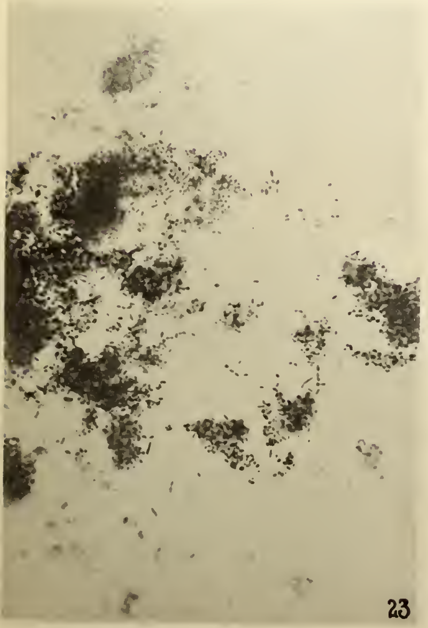
The microorganism isolated from human trachoma lesions (*B. granulosis, n. sp.*) and found capable of inducing chronic granular conjunctivitis in *Macacus rhesus* and the chimpanzee.


21

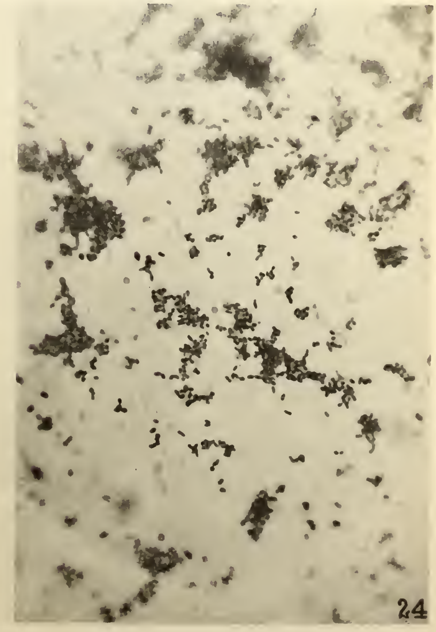
Four day culture on leptospira medium.


22

Culture stained by Gram's method, counterstained with fuchsin. × 1000.


23

Culture stained by Gram's method, counterstained with fuchsin. × 1000.


24

Culture stained with Giemsa's solution. × 1000.

Plate cultures of *B. granulosis* grown for six days at 30°C.

25

Rabbit blood agar.

26

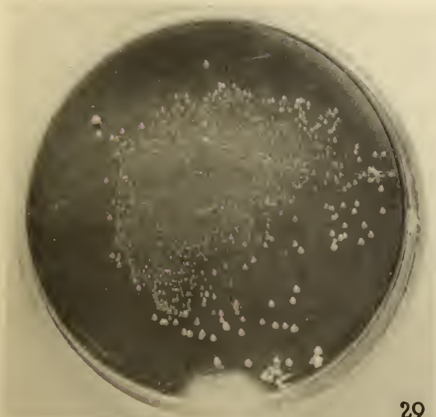
Rabbit blood agar containing sugars.

27

Horse blood agar.

28

Horse blood agar containing sugars.



29

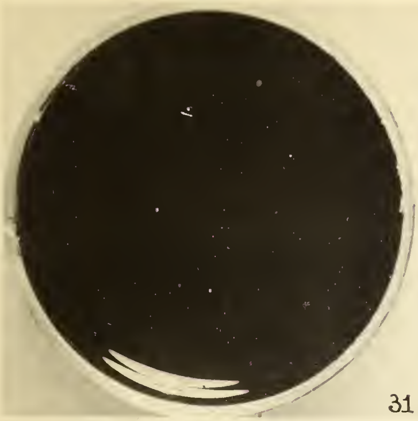
Solid leptospira medium.

30

Plain agar control.

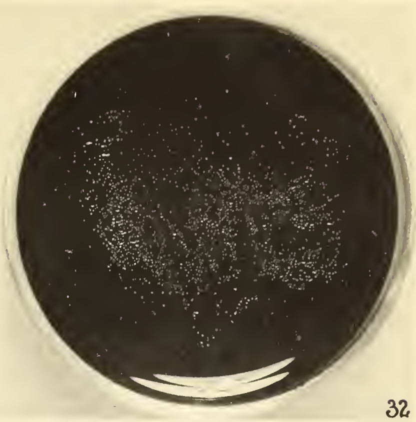
(Noguchi: Etiology of trachoma.)

Plate cultures of *B. granulosis* grown for six days at 37°C.



31

Rabbit blood agar.



32

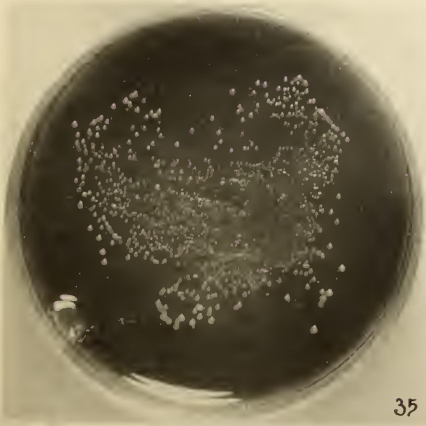
Rabbit blood agar containing sugars.

33

Horse blood agar.

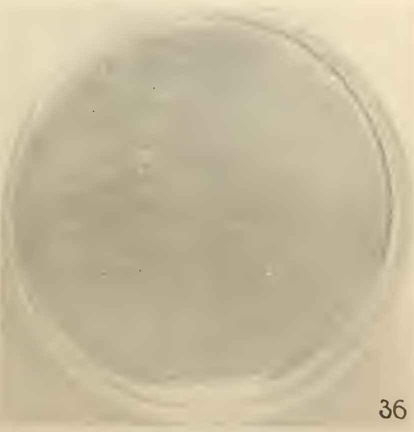
34

Horse blood agar containing sugars.



35

Solid leptospira medium.

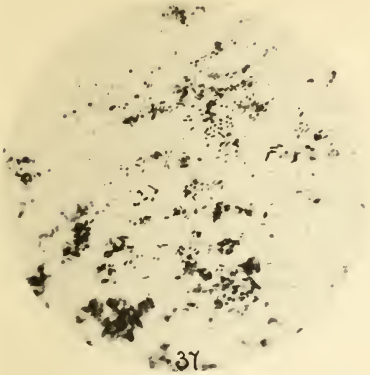


36

Plain agar control.

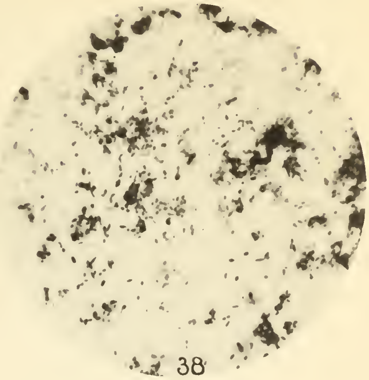
(Noguchi: Etiology of trachoma.)

Cultures of *B. granulosis* grown on leptospira medium (Figs. 37-40) and on horse blood agar (Figs. 41-42).



37

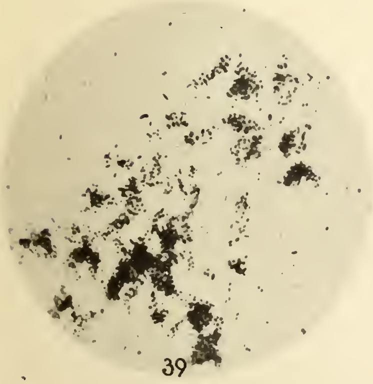
at 30°C.



38

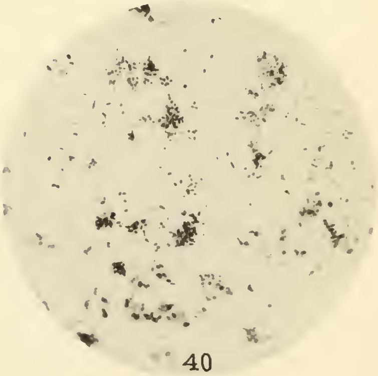
at 37°C.

Six day culture, Giemsa's stain. × 1000.



39

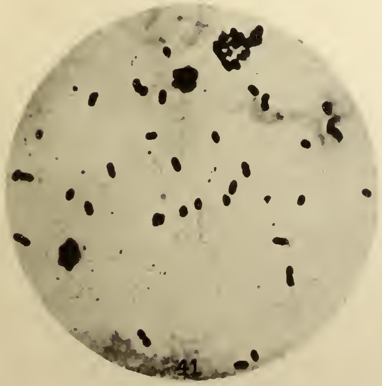
at 30°C.



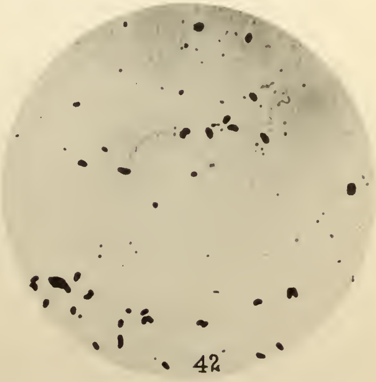
40

at 37°C.

Six day culture, Gram's stain, counterstained with fuchsin. × 1000.



41



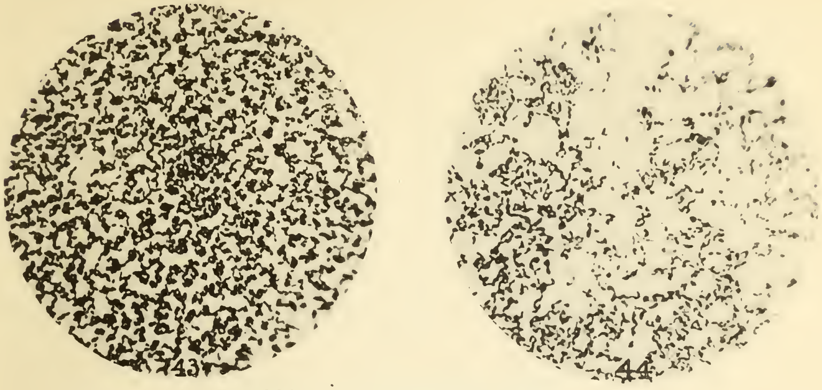
42

Motile culture, Zettnow-Fontana stain for flagella. × 2000.

(Noguchi: Etiology of trachoma.)

Cultures of *B. granulosis* grown

at 30°C. at 37°C.



On rabbit serum hemoglobin slant.

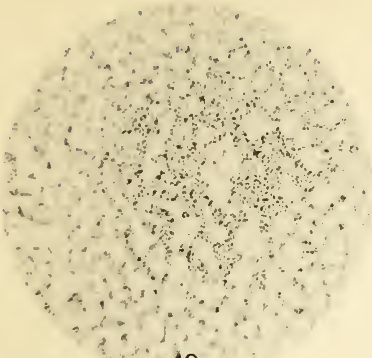
On rabbit plasma hemoglobin slant.

On rabbit plasma agar slant.

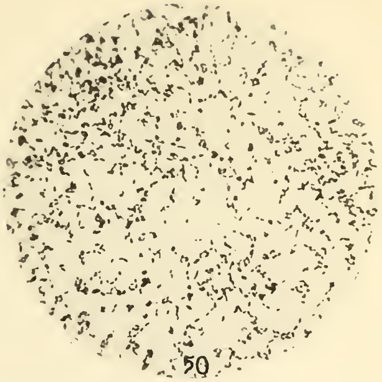
Magnification ×1000. Gram's stain, counterstained with fuchsin.

Cultures of *B. granulosis* grown

at 30°C. at 37°C.

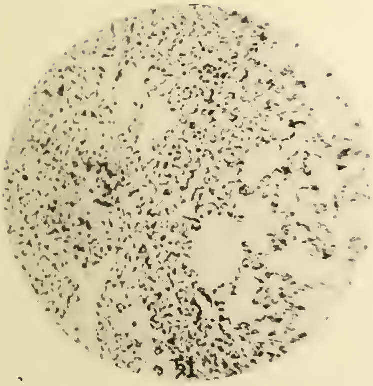


49



50

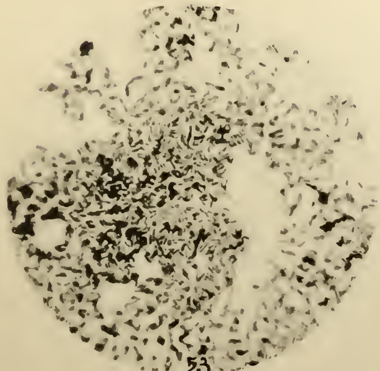
On horse serum hemoglobin slant.



51

52

On horse plasma hemoglobin slant.



53

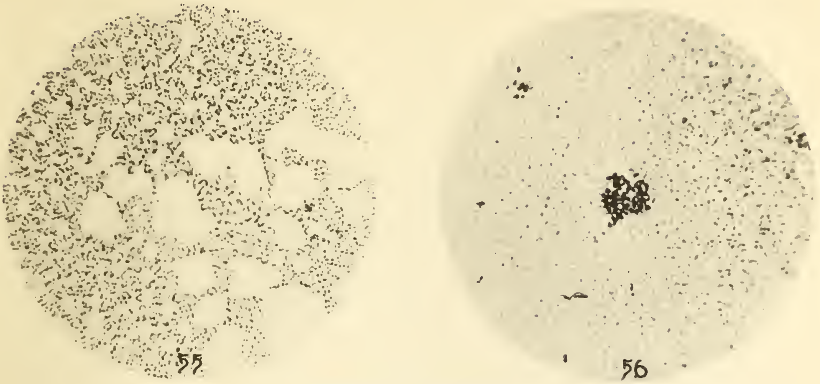
54

On horse plasma agar plate.

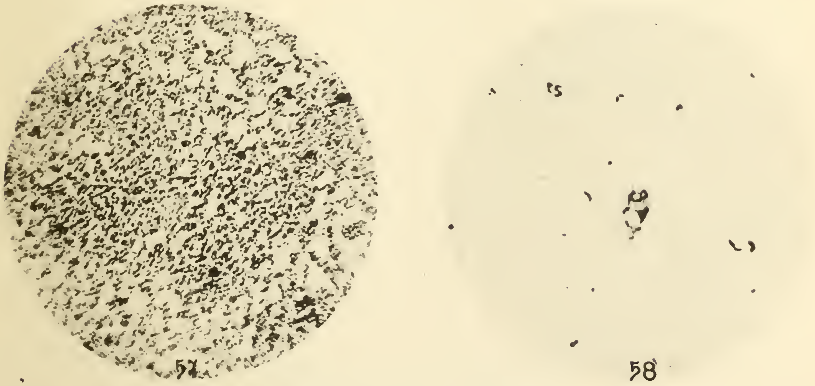
Magnification ×1000. Gram's stain, counterstained with fuchsin.

Cultures of *B. granulosis* grown

at 30°C. at 37°C.



On horse blood sugar slant.



On horse hemoglobin agar slant.

On rabbit serum agar slant.

Magnification ×1000. Gram's stain, counterstained with fuchsin.

PART II.

EXPERIMENTAL PRODUCTION OF CHRONIC GRANULAR CONJUNCTIVITIS IN MACACUS RHESUS AND CHIMPANZEE WITH BACTERIUM GRANULOSIS, N.SP.

The failure of direct transmission experiments with human trachomatous materials did not necessarily indicate that the specific microorganism was absent from the materials used for inoculation, or that the animals were absolutely refractory to trachoma. The investigations of Hess and Römer,[20] Morax,[21] Nicolle, Cuénod, and Blaizot,[28] Axenfeld,[23] Greeff,[24] and many others have definitely established the possibility of transmitting trachoma to certain monkeys, notwithstanding the naturally high resistance of these animals to the disease. Moreover, it is possible that, given the specific microorganism, inoculation of cultures might be more effective than that of human materials, since the number of organisms injected would be large. With this idea in mind, we concentrated on obtaining in pure culture every microorganism obtained from the cases of trachoma studied, in order that each might be tested separately on the conjunctiva of monkeys. As stated, five groups of microorganisms were isolated in pure culture from the Albuquerque cases of trachoma, namely, staphylococcus, *Corynebacterium xerosis*, sarcina, and two varieties of minute, Gram-negative bacilli. Subsequently several strains of the Koch-Weeks bacillus (*Hemophilus conjunctivilidis*) were cultured from other cases of Indian trachoma.[37]

It might seem superfluous to test on the conjunctiva such commonplace microorganisms as staphylococci, the *xerosis* bacillus, and the Koch-Weeks bacillus, which are known to be unrelated to trachoma. However, in our investigation, morphological and cultural characteristics were disregarded, in order to avoid overlooking any pathogen which might produce inoculation effects. Moreover, we thought it desirable to become familiar with the reaction induced in the conjunc-

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[37] These strains were obtained from materials kindly sent me by Dr. P. Richards, of Fort Defiance, Ariz., and Dr. C. H. Halliday, of the California Department of Health.

tiva by the several microorganisms in order that the specific lesions, if produced, might be recognized by contrast. As the experiments to follow show, indeed, one microorganism only of the six tested proved capable of inducing lesions resembling those of human trachoma. Hence the animals inoculated with the other five varieties served as valuable controls.

Method of Inoculation.—The conjunctiva of one eye was everted[36] and about 0.2 cc. of the culture suspension was injected with a tuberculin syringe into the subconjunctival tissues near the upper border of the tarsus. The tarsal conjunctiva was also lightly scratched and punctured with the point of the charged needle. The uninoculated eye served as a control.

Cultures 2 to 7 days old, grown either on leptospira medium, blood agar slants, or, in the case of the commoner organisms, on plain agar slants, were suspended heavily in saline solution. It was usual to combine several strains of the same organism for the injection. More than one animal was used, whenever possible, for testing each kind of microorganism. Since the results of the inoculations were likely to require several months to become apparent, 13 successive inoculations were made at short intervals (June 8, 12, 21, 24, July 3, 6, Sept. 18, Oct. 4, 16, 19, Nov. 12, 15, 18, 1926) to guard against attenuation or loss of virulence of the organisms due to delay and artificial cultivation. The motile Gram-negative bacilli and the sarcinas were inoculated into the larger number of animals, and it is of interest to remark that the bacterium which alone proved to be capable of producing granular or follicular conjunctivitis was at first regarded as of minor significance, and hence fewer animals were originally employed in testing its pathogenicity.

Inoculation of Non-Specific Bacteria.

Corynebacterium xerosis.—Four strains from the Albuquerque cases were pooled and injected into two *rhesus* monkeys (Nos. 8 and 8A) on June 12, 1926. Neither animal showed significant reaction.

Staphylococcus.—Three strains from the Albuquerque cases and one from *Macacus rhesus* 5 were inoculated into three *rhesus* monkeys (No. 7A on June 12, 1926, and Nos. 11A and 13A on Sept. 18, 1926). All reacted with acute purulent conjunctivitis without follicle formation. The inflammation subsided and normal conditions were restored in about 2 weeks.

Gram-Negative, Motile, Chromogenous Bacillus.—Four strains from the Albuquerque cases were injected on several occasions, either alone or together with sarcinoids, into ten *rhesus* monkeys, two chimpanzees, and one ourang-utan. A mild subacute mucopurulent conjunctivitis without folliculosis followed, ending in recovery in 2 to 3 weeks. The tabulation records this experiment.

1926				
June 12	*M. rhesus* 6	4 strains	Left eye	Acute conjunctivitis, no follicles
“ 21	“ “ 3	1 strain	Right “	“ “
“ “	“ “ 4	“ “	“ “	“ “
“ 24	“ “ 11	4 strains and 17 strains of sarcinoids	“ “	“ “
“ “	Chimpanzee “Jimmie”	“ “	“ “	“ “
July 3	Chimpanzee “Kitty”	“ “	“ “	“ “
“ “	*M. rhesus* 12A	“ “	“ “	“ “
“ “	Ourang-utan	“ “	“ “	“ “
“ 6	*M. rhesus* 12B	“ “	Left “	“ “
Oct. 4	“ “ 15	4 strains	“ “	“ “
“ “	“ “ 18	“ “	“ “	“ “
“ “	“ “ 19	“ “	“ “	“ “
“ “	“ “ 20	“ “	“ “	“ “

Sarcina.—Four strains from the Albuquerque cases, five from the Fort Defiance cases,[37] five from the Imperial Valley cases,[37] and two from *Macacus rhesus* Nos. 9 and 13 were injected on different occasions during a period of 5 months (June 8 to Nov. 18, 1926). Inoculations were made into one eye in fifteen and into both eyes in five animals. The rule was for mild swelling and congestion of the inoculated lid and conjunctiva to develop within 24 to 48 hours, after which gradual subsidence occurred, the conjunctiva returning to normal in a few weeks. Folliculosis was consistently absent. The tabulation gives the result of this experiment.

1926				
June 8	*M. rhesus* 1	4 Albuquerque strains	Left eye	—
“ “	“ “ 2	“ “ “	“ “	—
“ “	“ “ 4	“ “ “	“ “	—
“ 12	“ “ 7	“ “ “	“ “	—
Sept. 18	“ “ 11	“ “ “	“ “	—
“ “	“ “ 13	“ “ “	“ “	—
Oct. 4	“ “ 14	“ “ “	Right “	—
“ “	“ “ 15	“ “ “	“ “	—
“ “	“ “ 16	“ “ “	“ “	—
“ “	“ “ 17	“ “ “	“ “	—
“ “	“ “ 18	“ “ “	“ “	—
“ “	“ “ 19	“ “ “	“ “	—
“ “	“ “ 20	“ “ “	“ “	—
“ 19	“ “ 27	2 Fort Defiance “	“ “	—
“ “	“ “ 28	1 Imperial Valley strain	Both eyes	—
“ “	“ “ 29	*M. rhesus* 13 strain	Right eye	—
Nov. 18	“ “ 33	“ “ “ “	Both eyes	—
“ “	“ “ 34	“ “ 9 “	“ “	—
“ “	“ “ 35	3 Fort Defiance strains	“ “	—
“ “	“ “ 36	5 Imperial Valley “	“ “	—

Hemophilus conjunctivitidis (*Koch-Weeks Bacillus*).—Two strains from the Fort Defiance cases and one from the Imperial Valley cases were inoculated into five monkeys without noticeable effect, as shown in the following tabulation.

1926					
Oct. 16	*M. rhesus* 23	Fort Defiance	2 strains	Right eye	—
" "	" " 24	" "	" "	" "	—
" 19	" " 26	Imperial Valley	1 strain	Both eyes	—
Nov. 12	*M. cynomolgus* 30	" "	" "	Right eye	—
" "	" " 31	" "	" "	" "	—

Reviewing these inoculations, it may be stated that of 43 monkeys injected, of which 38 were *Macacus rhesus*, 2 *M. cynomolgus*, 2 chimpanzees, and 1 ourang-utan, none yielded follicle formation, even though several showed mild or severe grades of conjunctivitis. Hence we conclude that the conjunctiva of the animals used does not react with folliculosis to inoculation of the several bacteria mentioned, irrespective of whether they are injected into, or merely applied to, the injured tarsal conjunctiva.

Inoculation of a Specific Microorganism.

The results were very different when cultures of the non-chromogenous Gram-negative bacillus, *Bacterium granulosis*, *n. sp.*, were inoculated. As will be recalled, this bacterium grows only on particular culture media. A single monkey (*M. rhesus* 5) was inoculated on June 12, 1926, and another (*M. rhesus* 8) on June 21. Both animals showed lesions regarded as suspicious in July. By early September both animals showed numerous granules or follicles along the border of the tarsus and fornix of the upper lid, and some scattered follicles on the tarsal conjunctiva and the lower lid. Moreover, Monkey 5 showed follicles on the upper lid of the uninoculated side. Before injection of the culture, the conjunctivæ had been perfectly smooth. The conditions met with in these animals were entirely unlike those observed in the animals inoculated with the other microorganisms.

In order to exclude tuberculosis, scrapings of the conjunctival lesions were inoculated intraperitoneally into guinea pigs and corneally into the anterior chamber of the eyes of rabbits. The results were negative, while control animals injected with the lung lesion of a tuberculous *rhesus* monkey developed typical tuberculous lesions within a month. In order to compare the lesions produced by the culture with tuberculous lesions of the conjunctiva, two *rhesus* monkeys (Nos.

58 and 59) were inoculated into the conjunctiva with a suspension of tuberculous lung tissue. Within 3 weeks diffuse general infiltration developed, involving the entire conjunctival sac and accompanied by swelling and induration of both upper and lower lids and a yellow discharge. The bulbar conjunctiva also became involved, and general tuberculosis supervened. The comparison made it certain that the lesions in Monkeys 5 and 8 were not of the nature of tuberculous conjunctivitis.

Two *Macacus rhesus*, Nos. 9 and 10, were injected on September 18, 1926, with cultures of the same organism used in Monkeys 5 and 8. At the time four normal *M. rhesus* were inoculated, two each with sarcinas and staphylococci. Monkey 9 developed the same type of lesions as had Monkeys 5 and 8, while Monkey 10 did not. The other four monkeys showed various grades of acute purulent conjunctivitis, which healed within 2 weeks. The difference in result was striking and decisive.

On November 15, 1926, *M. rhesus* Nos. 120 and 32 were inoculated with the bacillus, and 3 days later four *rhesus* were injected with cultures of sarcinas. The differences were again striking: Monkeys 120 and 32 developed granular conjunctivitis, which was especially severe and persistent in Monkey 32, while the other four animals showed merely mild transient conjunctivitis.

On January 26, 1927, fresh subcultures of the same bacterium, which had then been under cultivation for more than 6 months, were injected into five *rhesus* monkeys (Nos. 46, 47, 48, 49, and 50). After an incubation period of 2 to 3 weeks a mild granular conjunctivitis appeared which receded within a few weeks.

The earlier bacterial cultures (3rd, 4th, and 5th generations), which had been preserved in the refrigerator (4°C.) for several months, were tested on February 8, 1927, at the same time as fresh subcultures. *Rhesus* 52 and a chimpanzee, "Marie," (Plate 14, Figs. 5–6) were injected with the mixed early cultures. The *rhesus* developed a marked granular conjunctivitis which lasted about 3 months. The chimpanzee reacted differently. Numerous small, glistening, discrete prominences appeared on the tarsal conjunctiva and fornix of the inoculated eye within about 8 days, and after a week similar but less numerous follicles appeared on the uninoculated lid. There was little congestion of the conjunctiva. The granular condition persisted and slowly increased. 4 months later the granules were much larger,

TABLE V.

Macacus rhesus No.	Inoculations of cultures		Results of inoculations				Remarks
	Date of inoculation	Site of inoculation	Inoculated side		Not inoculated side		
			Upper	Lower	Upper	Lower	
	1926						
5	June 12	Right upper	++++	+	++++	+	Died in 8 mos.
8	" 21	" "	++++	+	–	–	Scars "7 "
9	Sept. 18	Left "	++++	++	+++	+	Stationary after 8 mos.
10	" "	" "	–	–	–	–	
32	Nov. 15	" "	++++	+	++	+	Still progressing at time of death from tuberculosis (296 days)
120	" "	Right "	+	–	±	–	Died of tuberculosis
37	" 20	Left "	+	<+	+	<+	" "
	1927						
46	Jan. 26	Right "	<+	–	–	–	Receded in 2 mos.
47	" "	" "	±	<+	–	–	Receded in 3 mos.
48	" "	" "	±	–	–	–	Receded in 2 mos.
49	" "	" "	+++	–	–	–	Died of tuberculosis
50	" "	" "	±	–	–	–	Receded in 1 mo.
52	Feb. 8	Left "	++	<+	–	–	Receded in 3 mos.
Chimpanzee "Marie"	" "	" "	+	<+	+	<+	Persisted 7 mos.
72	June 7	" "	++++	++	++++	++	Still well marked
73	" "	" "	++++	++	++++	++	" " "
Macacus speciosus 2	" "	" "	+	+	–	+	Stationary. Few follicles on lower lids

<+ Slight but definite granules receding in about 2 months.
± Transient granules persisting for several weeks only.
++++ Very extensive lesions showing no retrogression in 8 months.
+++ Less extensive lesions becoming stationary in about 4 months.
++ Moderate lesions receding in about 3 months.
\+ Mild lesions lasting 2 months or longer.

the lid was slightly congested, and the conjunctiva appeared thickened at the border of the tarsus and fornix. After 7 months a mild follicular conjunctivitis was still present. It will be recalled that the injection of the chromogenous Gram-negative bacillus and of sarcinoids produced no follicles in the conjunctivæ of two other chimpanzees which were observed for a period of 6 months.

The earlier cultures were again tested, together with fresh subcultures, and cultures recovered from a chimpanzee of the 3rd passage (see page 35), on June 7, 1927. Two *rhesus* monkeys (Nos. 72 and 73) both developed a granular conjunctivitis which was slow in appearing but is of a very severe and striking type and is still well marked (May 25, 1928). A Japanese monkey (*M. speciosus* 2) showed a mild reaction, and still has a few follicles on the lower lids.

In Table V are recorded the inoculation results with the bacterium. The protocols of individual animals follow.

M. rhesus 5 (Plate 14, Figs. 1–4), inoculated in the right eye on June 12, 1926, with pure culture of the 2nd generation, showed slight traumatic reaction, subsiding in a few days, immediately following the inoculation. 13 days after inoculation the conjunctiva was congested and somewhat edematous, and grayish granules were apparent along the conjunctiva near the everted edge of the tarsus and in the fornices near both canthi. The uninoculated (left) lid also showed a few follicles, indicating an autoinfection, the lids having been absolutely free from any granules before inoculation. The inflammation progressed rather rapidly until after 2 or 3 months the granules invaded half the tarsal surface from the cul-de-sac in both eyes. This monkey succumbed to tuberculosis at the end of 7 months, when the lesions were still active and the lower lids were becoming involved.

M. rhesus 8 (Plate 15, Figs. 7–12), inoculated in the right eye on June 21, 1926, with a culture of the 3rd generation, reacted much more slowly than the previous animal, and the infection remained confined to the inoculated side until 173 days later, when the left eye was experimentally inoculated with material removed from the right eye. Within 10 days the left eye showed severe congestion and edema of the conjunctiva, followed by numerous follicles in the following weeks. The lesions in the right eye (the first inoculated) progressed slowly and steadily, covering nearly half of the tarsal surface within 4 to 5 months, while those of the left eye, inoculated from the right, progressed more rapidly, involving the whole tarsal conjunctiva within about 4 weeks. The conjunctivæ of the lower lids were little affected. Towards the end of 7 months definite scar tissue fibrils were detectable on the tarsal surface of the right lid (Fig. 11) both by gross examination and on section. This animal died of tuberculosis 237 days after the first inoculation.

M. rhesus 9 (Plate 16, Figs. 13–16) was inoculated in the left eye on September 18, 1926, with a 4th generation culture of the same bacterium. No effects followed until 11 days later, when slight hyperemia and edema were noted in both eyes, and minute granules were observed on the tarsal border of the everted lids as well as in the fornices. The congestion of the right eye was less than that of the left. Lesions excised[36] from the right eye 37 days after inoculation revealed lymphoid infiltration of the subepithelial layer and numerous large follicles in the fornices. After 7 months the lesions of the upper and lower lids were still active. Numerous grayish follicles surrounded by dilated capillaries were present on the bulbar conjunctivæ of both eyes. This animal died of pulmonary tuberculosis on Oct. 14, 1927, 391 days after inoculation. Scar tissue formation was definite on the left upper conjunctiva.

M. rhesus 32 (Plate 16, Figs. 17–18) was inoculated with a 5th generation culture in the left eye on Nov. 15, 1926. No effects were manifest until 21 days later, when the inoculated side showed a row of numerous grayish granules along the tarsal border of the everted lid and in the fornices; a few granules were present on the lower lid. The right (uninoculated) conjunctiva was smooth at this time but became granular within 5 weeks. The number of follicles steadily but very slowly increased, until 5 months later they invaded a considerable part of the tarsal surface. After 8 months the conjunctivæ of both lids were congested and hypertrophied, and follicles extended over the entire surface, including even the tarsal region. The lesions were still progressing when the animal was etherized, because of pulmonary tuberculosis, 296 days after the inoculation. Microscopical examination of the sections showed that scar tissue had begun to form in the upper tarsal conjunctiva.

M. rhesus 48 and 49 were inoculated with fresh subcultures (8 days old) on Jan. 26, 1927. *M. rhesus* 49 showed signs of the development of granulation about 1 month after inoculation. These disappeared, but after 184 days, when the animal was found to be dying of pulmonary tuberculosis, examination showed numerous follicles on the lower lid of the right eye. *M. rhesus* 48 had no reaction.

M. rhesus 72. Left conjunctiva inoculated on June 7, 1927, with a mixture of cultures 1 year old (kept at 4°C.), fresh subcultures of May 29, 1927, and a culture recovered from a chimpanzee (3rd passage). A few follicles were seen 52 days after inoculation, and 73 days later there were numerous follicles on the left conjunctiva and a few on the right. On Nov. 4 (150 days after inoculation) the granulation was well marked in both retrotarsal folds, and there were some granules on both tarsi. On Dec. 9 a small piece of tissue was excised[36] from the left lid for examination and transfer. The lesions on the right side are still well marked (May 25, 1928) and involve both upper and lower lids.

M. rhesus 73. Duplicate of the foregoing experiment. The development of the lesions was even slower in this animal than in *M. rhesus* 72. On Nov. 22 (168 days after inoculation) the entire left retrotarsal fold was covered with follicles, and there was some granulation of the tarsal conjunctiva. A month later the uninoculated side was involved to some extent. On Feb. 9, 1928, the entire left

tarsus was excised[36] to furnish material for the inoculation of a group of *rhesus* monkeys. The lesions on the right lid are still (May 25, 1928) extensive. The lower lid on the left side shows granulation.

Plates 17–21 are intended to show the histological characters of the normal and the pathological conjunctivæ of *Macacus rhesus*. The infiltrating cells composing the pathological process, including the follicles, resemble closely those present in human trachomatous lesions. In the succeeding pages the histological changes will be described in detail.

SUMMARY.

A study has been made of the pathogenic action of several kinds of microorganisms cultured from cases of Indian trachoma. These pure cultures, in heavy suspension, have been injected into the subconjunctival tissues of *Macacus* monkeys and the chimpanzee.

With one notable exception, the cultures produced only fleeting reactions or acute inflammation, which soon subsided.

The exception is notable because the injection of the culture of a bacillus, called *Bacterium granulosis, n. sp.*, induced a persistent granular conjunctivitis resembling closely, and apparently identical with, trachomatous granular conjunctivitis in man.

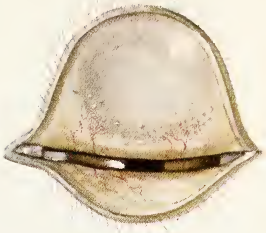
It is noteworthy that when the granular condition was induced in one eye by the injection, spontaneous infection, attended by granular conjunctivitis, followed in the other eye, and when the tissues from a slowly progressing granular conjunctivitis in one eye were removed and inoculated into the conjunctiva of the other side, a more rapidly advancing granular conjunctivitis of this side was produced.

The histological changes of the experimentally induced conjunctival lesions correspond closely with those of human trachoma and include the characteristic follicle and scar tissue formation.

Conjunctivæ of *Macacus rhesus* 5.

1

Right—17 days after inoculation with culture.

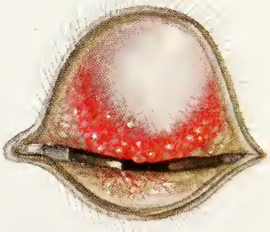


2

Left—which became infected from right.

3

Right—177 days later. (Tarsus removed 38 days previously.)



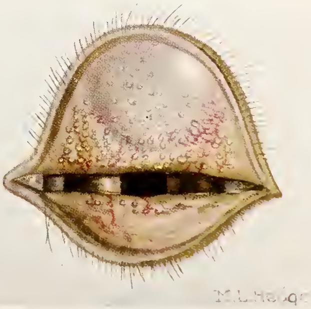
4

Left—139 days later.

Conjunctivæ of Chimpanzee "Marie."

5

Right—142 days after inoculation with culture (human strain).



6

Left—96 days after inoculation with culture (from Chimpanzee "Kitty").

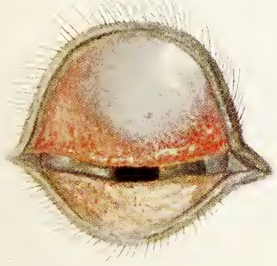
Conjunctivæ of *Macacus rhesus* 8.

7

Right—151 days after inoculation with culture.

8

Left—not inoculated.

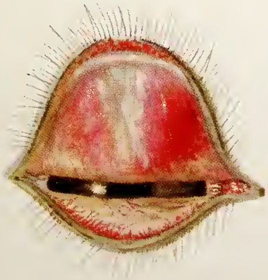


9

Right—196 days after inoculation.

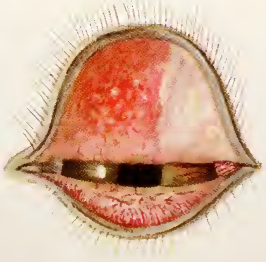
10

Left—24 days after inoculation from right.



11

Right—234 days after inoculation, showing cicatrization.



12

Left—62 days after inoculation (portion of tarsus removed 29 days previously).

(Noguchi: Etiology of trachoma.)

Conjunctivæ of *Macacus rhesus* 9.

13

Right—which became infected from left.

14

Left—62 days after inoculation with culture.

15

Right—47 days later (tarsus removed 72 days previously).

16

Left—109 days after inoculation (tarsus removed 24 days previously).

Conjunctivæ of *Macacus rhesus* 32.

17

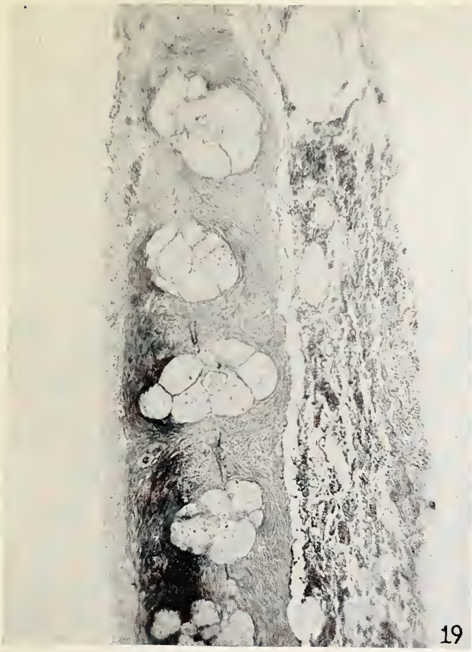
Right—which became infected from left.

M.L.Hedge 18

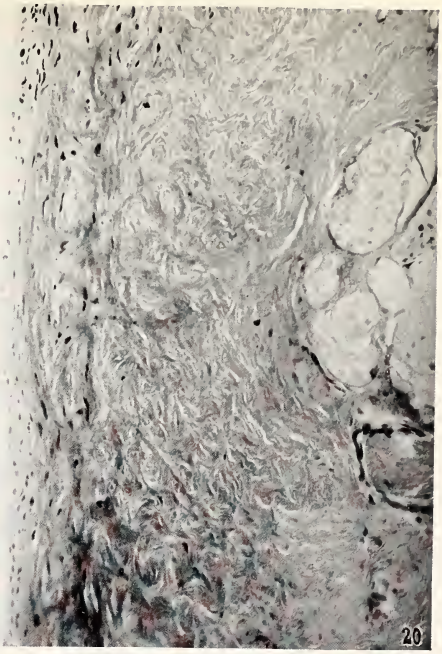
Left—227 days after inoculation with culture.

(Noguchi: Etiology of trachoma.)

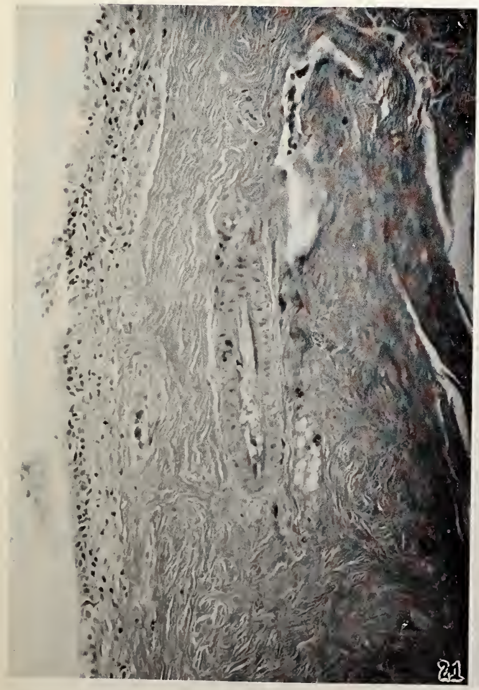
Normal conjunctiva (upper) of *Macacus rhesus*. Giemsa's stain.


19

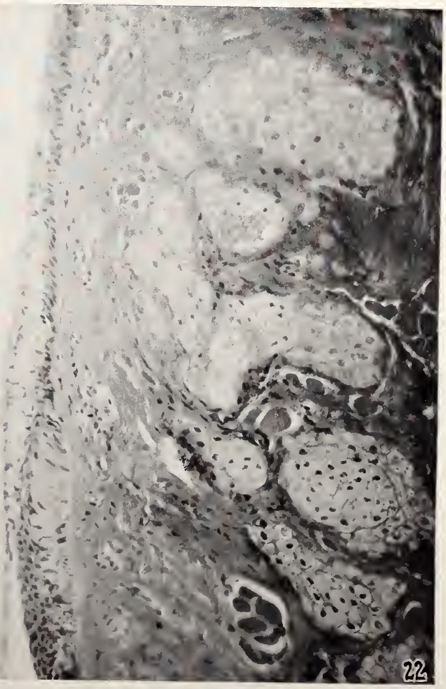
× 52.


20

× 156. Near the fornix.


21

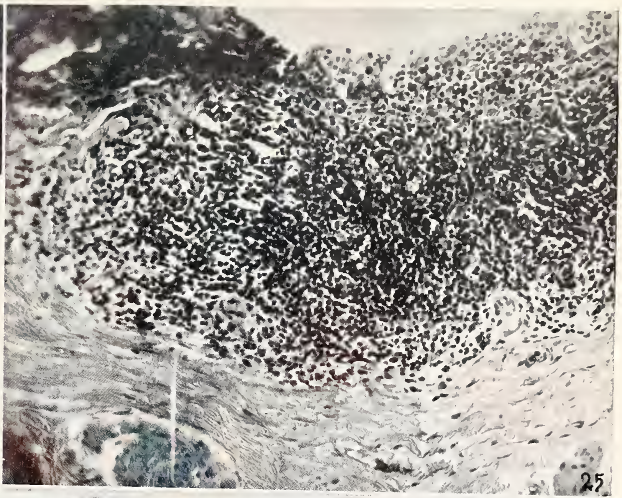
× 156. Middle tarsal region.

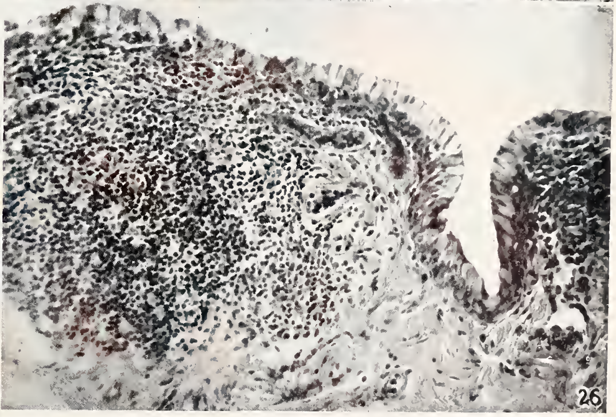

22

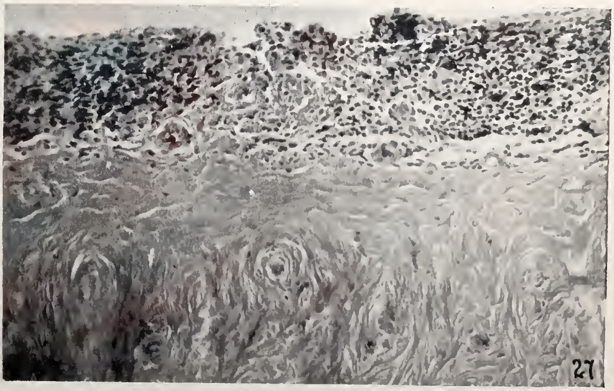
× 156. Lower tarsal region near the free edge of the lid.

Conjunctiva (right upper) of *Macacus rhesus* 5, 156 days after inoculation of culture. Giemsa's stain.

FIGS. 23–24. Follicle and lymphoid infiltration of retrotarsal and tarsal conjunctiva. × 52.

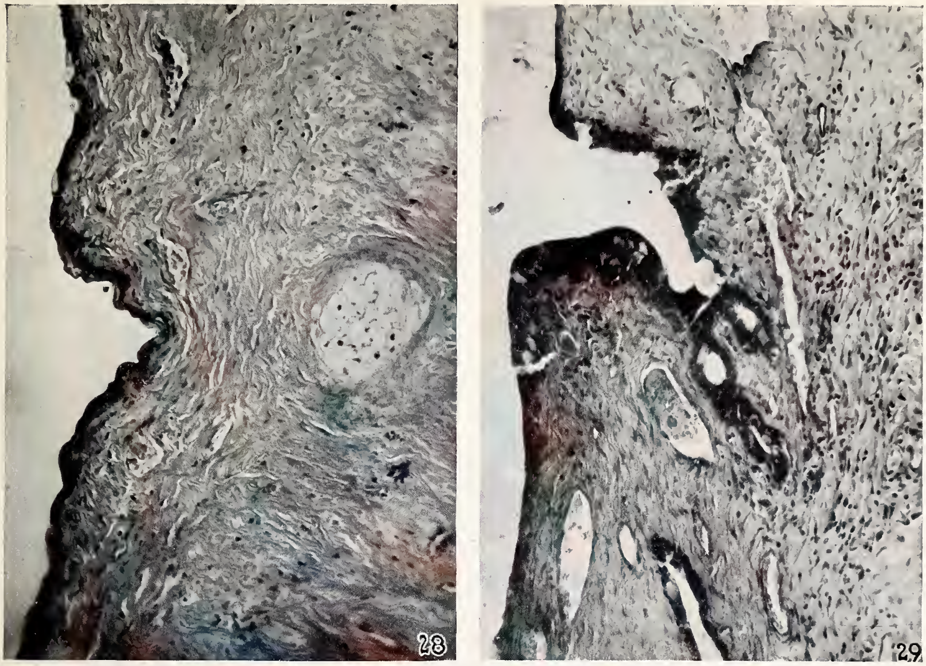




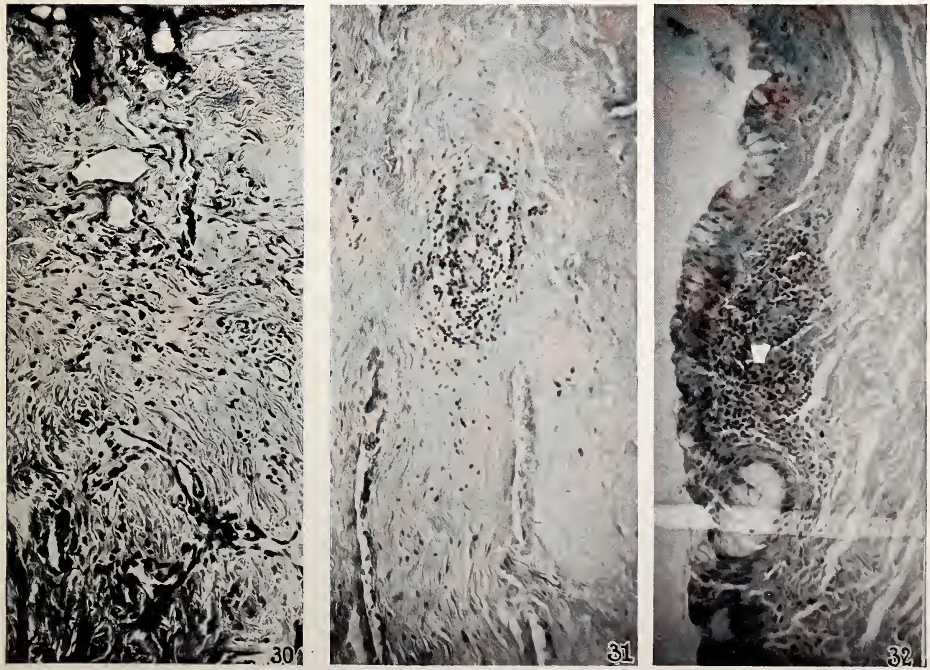


FIGS. 25–27. The same section, × 156, showing the character of the follicles and the infiltration.

Sclerotic changes of the conjunctivæ in the advanced stages of experimental infection in *Macacus rhesus*. Giemsa's stain. × 156.



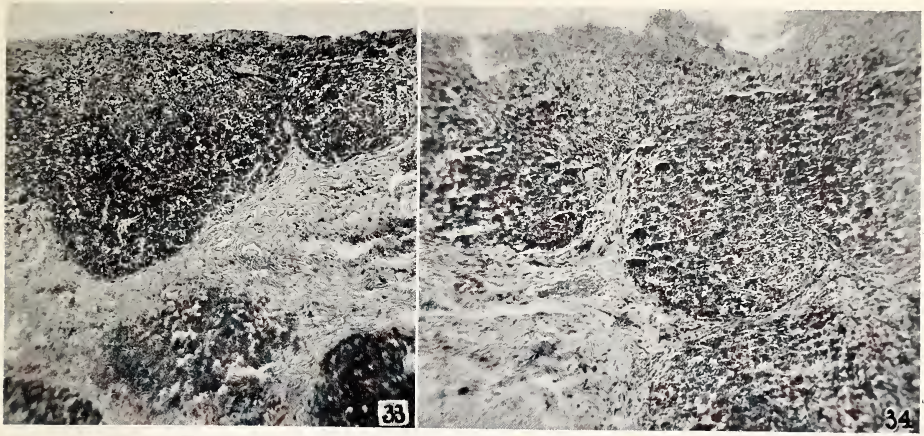
M. rhesus 8, 234 days after inoculation of culture. Right upper lid.



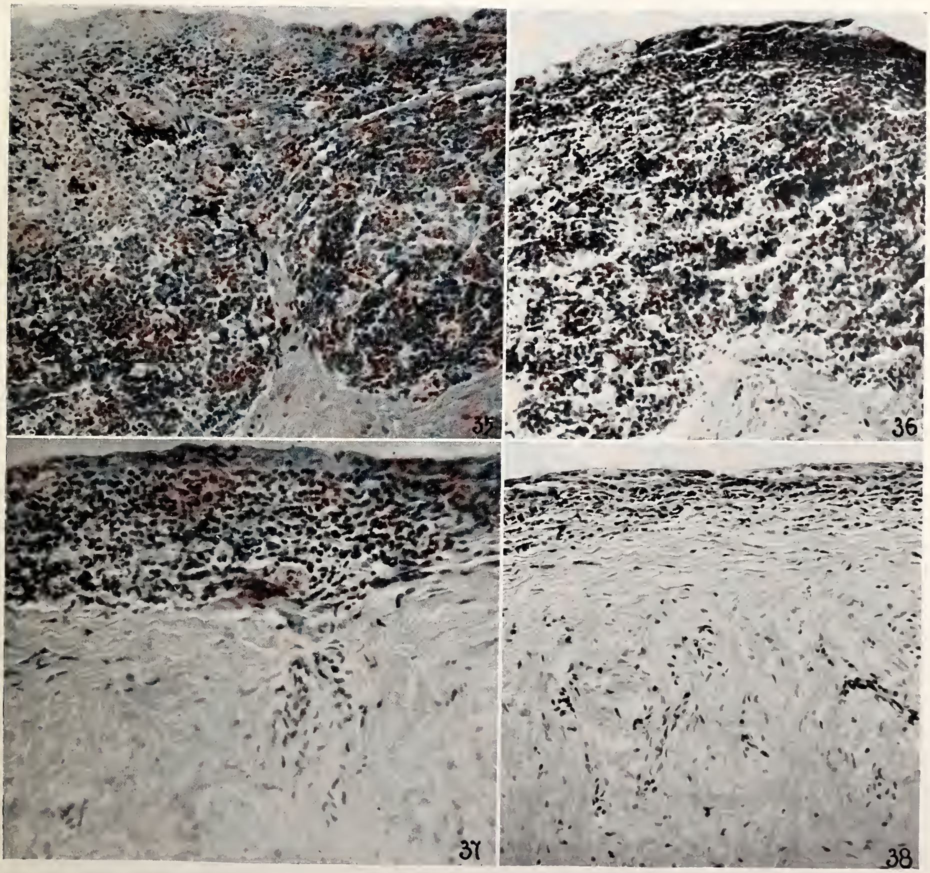
The left upper conjunctiva of the same monkey, tissue removed 65 days after transmission of the infection from the right eye.

(Noguchi: Etiology of trachoma.)

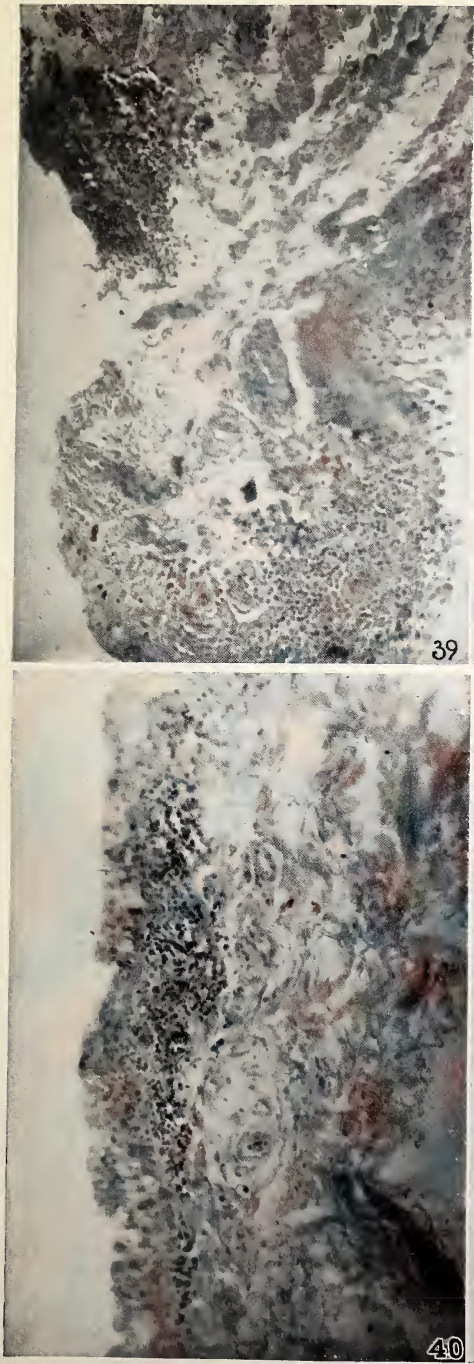
Sections of conjunctiva (right upper) of *Macacus rhesus* 9, 37 days after inoculation of culture. Giemsa's stain.



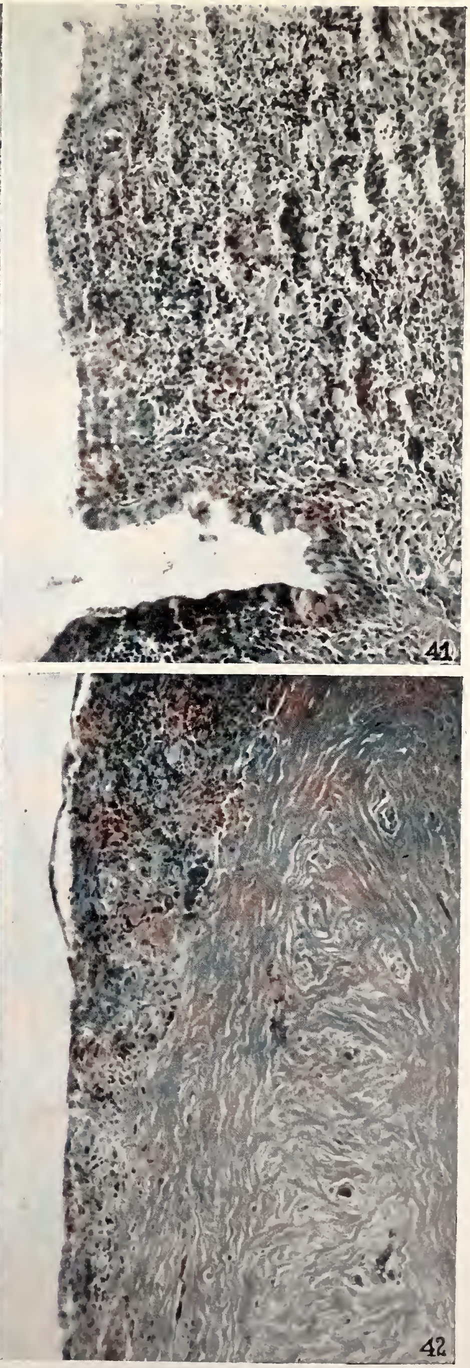
Figs. 33–34. Lymphoid infiltration and follicles. × 52.



Figs. 35–38. The same section, × 156, showing type of cells, structure of follicles, and diffuse cellular infiltration.



Figs. 39–40. Conjunctiva (left upper) of *M. rhesus* 8, 33 days after inoculation from the other eye. Giemsa's stain. × 156.



Figs. 41–42. Conjunctiva (right upper) of *M. rhesus* 32, 97 days after inoculation of culture. Giemsa's stain. × 156.

PART III.

TRANSMISSION OF EXPERIMENTAL GRANULAR CONJUNCTIVITIS FROM ANIMAL TO ANIMAL.

A bacillus isolated from cases of trachoma in Albuquerque, New Mexico, named *Bacterium granulosis, n. sp.*, having been proven capable of inducing a chronic granular conjunctivitis of varying severity in *Macacus rhesus* and the chimpanzee, the next step was to determine the possibility of direct transfer of the disease from monkey to monkey.

Transmission from M. rhesus 5.

This animal, inoculated with culture on June 12, 1926, had first shown the granular condition of the conjunctiva 17 days later. At this time a bit of the fornix was excised,[36] suspended in saline, and injected into two healthy *rhesus* monkeys.

M. rhesus 21, inoculated in the right upper lid on June 29, 1926, showed 4 weeks later a mild granular condition of the inoculated lid, the other lid being still normal. A month later the lesions had progressed somewhat, but after another month they were found receding. The histology of the lesion at its active stage was the same as that of *M. rhesus* 5.

M. rhesus 22 was inoculated in the same manner as *M. rhesus* 21. A few granules had developed in the course of 4 weeks. 4 months later the conjunctiva was normal.

To return to *M. rhesus* 5. On November 15, 1926, 156 days after the culture had been inoculated, marked lesions were present on both upper lids. A bit of the conjunctiva was removed[36] from the fornix of the left upper lid, suspended in saline, and injected into two *rhesus* monkeys.

M. rhesus 2, inoculated into the right upper conjunctiva on Nov. 15, 1926, showed within a month a moderate granular condition of the lid. Slight extension of the lesions took place, but the progress soon ceased, although they remained stationary for several weeks. Because of advanced tuberculosis, the animal was etherized 81 days after inoculation. The conjunctival lesions at this time were histologically identical with those of *M. rhesus* 5.

M. rhesus 3 was inoculated into the left upper lid as was the foregoing monkey. No effects followed.

Although the lesions produced were far from pronounced, these tests indicated the possibility of the passage of granular conjunctivitis from monkey to monkey by means of tissues taken from the lesion as early as 17 days and as late as 156 days after the culture inoculation.

Transmission from M. rhesus 8.

M. rhesus 8, inoculated with the culture on June 21, 1926, developed a moderately severe granular conjunctivitis on the inoculated side lasting until the death of the animal, 234 days after the inoculation. The other conjunctiva was unaffected.

The first transfer was made into two *rhesus* on December 10, 1926, with tissue excised[36] 172 days after the inoculation.

M. rhesus 66 developed within 4 weeks in the inoculated conjunctiva a mild granular lesion lasting 3 months but completely disappearing.

M. rhesus 65 was inoculated in the same manner as the foregoing animal but without result.

The 2nd transfer from *M. rhesus* 8 was made on January 12, 1927, 204 days after inoculation, two *rhesus* monkeys being inoculated.

M. rhesus 44 died of tuberculosis a month and a half after inoculation. It showed mild lesions which did not appear to be actively progressing at the time of death.

M. rhesus 45, inoculated in the same manner as the foregoing animal, developed in the uninoculated as well as the inoculated lid a marked granular condition which was still progressing when the animal died of pulmonary tuberculosis 149 days after inoculation.

Second Passage from M. rhesus 8.—Several transfers made at various periods with tissue from the lesions of *M. rhesus* 45 yielded only three positive results among fourteen mature *rhesus* monkeys. A baboon and an ourang-utan also failed to react to the inoculation of this material. Another baboon developed characteristic lesions which persisted for 6 months and then receded. Notwithstanding the high percentage of negative results of the transmission experiments, the histological picture in the case of *M. rhesus* 45 was strikingly characteristic (Plate 22).

M. rhesus 58 was inoculated on Mar. 22, 1927, with tissue from the 69 day old lesion of *M. rhesus* 45. Definite granulation in the inoculated (left) eye is first recorded for this monkey on Apr. 15, that is, 24 days after inoculation. The lesions, which were present on the tarsus and lower lid, as well as the conjunctiva, progressed rather rapidly and by May 16 were very striking. The animal succumbed to pulmonary tuberculosis on July 6.

M. rhesus 64, inoculated in the same manner as the foregoing animal, exhibited mild lesions 28 days after inoculation. The condition appeared to progress slowly for a period of 3 months but then disappeared.

Transmission from M. rhesus 9.

This animal was inoculated with the culture on September 18, 1926, on the left side, and was the third to develop granular conjunctivitis, first on the inoculated, and then on the uninoculated conjunctiva. Transfers were made 37 days and 83 days after the inoculation in the manner described for *M. rhesus* 5.

M. rhesus 30. Inoculation into both eyes on Oct. 25, 1926, was followed by granular conjunctivitis in 19 days, and in 56 days the lower as well as the upper conjunctiva was involved (Plate 23, Figs. 5–6). This animal died from the effects of ether while under anesthesia for the excision of lesions for transfer and histological examination. The histological character and localization of the follicular lesions are identical with those induced by direct injection of the culture (Plate 24, Figs. 11–14).

Two *rhesus* monkeys (Nos. 38 and 39) were inoculated from *M. rhesus* 9 when the lesions were 83 days old. Only one reacted with mild lesions, which receded in a few months.

Second Passage from M. rhesus 9.—The transfers from *M. rhesus* 30 to other *rhesus* and to chimpanzees are of particular interest, since they brought out the important fact that the chimpanzee may be susceptible to inoculation. Another point brought out is that a *rhesus* monkey may escape infection with material which is infective for a chimpanzee.

Chimpanzee 1, "Kitty" (Plate 23, Figs. 7–10). Left upper conjunctiva injected Dec. 20, 1926, with a saline suspension of the follicular tissue removed from the upper lids of *M. rhesus* 30, inoculated 56 days previously. 4 weeks later granular conjunctivitis was present in the chimpanzee. The lesions slowly but steadily progressed until they covered about one-third of the tarsal region and the entire fornices of both upper and lower lid (Figs. 8, 10). The right (uninoculated) eye became involved 4 months after the left eye and 2 months later showed definite

granular conjunctivitis (Fig. 9). After the lapse of 10 months, an irregular area, about 2 mm. wide horizontally and 4 mm. long vertically, adjacent to the inner tarsal border and canthus of the left upper conjunctiva, became definitely scarred. No pannus has so far developed.

M. rhesus 43, similarly inoculated, showed mild lesions which subsided in a few months; *M. rhesus* 42 made a doubtful response.

Chimpanzee "Jimmie," similarly inoculated, developed a moderate granulation which receded within about 4 months.

The appearance of the conjunctivæ of the chimpanzee "Kitty" recalls that of some of the human cases of trachoma seen in Albuquerque, as may be seen from the illustrations. There is definite increase in connective tissue fibrils in the affected areas. The histological structure of the lesions in the fornix near the external canthus of the left upper lid, a portion of which was excised[36] 193 days after inoculation, was found to be identical with that of *rhesus* monkeys. Plate 25 shows the normal conjunctivæ of a chimpanzee, while Plates 26–28 show the experimental lesions of the chimpanzee "Kitty." As will be noted, some of the follicles are invaded by fine connective tissue fibrils (Plate 27, Figs. 18–19).

Third Passage from M. rhesus 9.—Transfer was made from Chimpanzee "Kitty" to a *Macacus rhesus* on February 8, 1927, when the lesion was 49 days old, and to another chimpanzee, "Louisa," on May 20, 1927, when the lesions were 151 days old.

The *rhesus* (No. 51) developed characteristic lesions, first in the left (inoculated) lid and later also in the other eye. The condition progressed steadily for 5 months, when the animal died of pulmonary tuberculosis.

The chimpanzee "Louisa" first showed a moderate granular condition of the inoculated lid about 4 weeks after the inoculation. On July 29, 70 days after the inoculation, the lesions had extended over the tarsal surface of the inoculated side and had appeared in the uninoculated eye. After 8 months the lesions had progressed, especially in the upper conjunctivæ, the granules being almost confluent over nearly half of the tarsal region. A bit of the fornix near the inner canthus of the left upper lid was excised[36] 146 days after the inoculation for further transfers and for histological examination. As shown in Plate 29, Fig. 22, the microscopic characters are typical of trachoma. On May 25, 1928, there is still well marked infiltration of the retrotarsal fold and about half of the tarsus. There are typical trachomatous follicles on both upper and lower lids, with characteristic obliteration of blood vessels.

Rhesus Monkeys 67 and 68, inoculated with the lesion of chimpanzee "Kitty"

on May 20, 1927, both developed granular conjunctivitis in the course of several weeks. The lesion spread from the inoculated to the uninoculated eye within 3 months. The histological structure of the lesion excised from *M. rhesus* 67,[36] 118 days after the inoculation, for examination and transfer, is presented in Plate 29, Figs. 23–24.

Fourth Passage from M. rhesus 9.—Transfers were made from *M. rhesus* 67 on September 15, 118 days after inoculation, to two *rhesus* monkeys (Nos. 74, 75). The inoculation yielded no results in either animal.

On October 13 a small piece of tissue was removed[36] from the left upper tarsal conjunctiva of the chimpanzee "Louisa." Two chimpanzees, "Venus" and "Adonis," were inoculated with this material and also five *rhesus* monkeys. One chimpanzee, and all except one *rhesus*, developed typical lesions. The observations recorded for these animals have been confirmed by Dr. Proctor (December 1, 1917), by Drs. Knapp and Friedenwald (January 26, 1928), by Dr. Cohen (March 9, 1928), and by Drs. Proctor, Richards, and Posey on May 25, 1928.

The characteristic granular conjunctivitis was noticed at about the same time (22 days after inoculation) in Monkeys 78, 79, and 80, and by the 40th day was well marked in all three, though somewhat more severe in Monkey 80 than in the others. The lesions are still progressing (May 25, 1928), and in Monkey 80 the left upper lid shows definite thickening and some contraction, suggesting scar tissue formation.

M. rhesus 81 developed the lesions in both eyes simultaneously 89 days after the inoculation. The follicles are very numerous in the retrotarsal folds in both eyes, and are present on both tarsi.

M. rhesus 82 showed no response to the inoculation.

Chimpanzee "Venus" had developed mild but definite lesions on the inoculated lid when seen by Dr. Proctor on Dec. 1, 1927 (49 days after inoculation). At the time of writing (May 25, 1928) there are numerous granules in both folds and a few follicles on the tarsi. Both lower lids show well marked infiltration, with obliteration of the blood vessels by the follicles.

The other chimpanzee died of pneumonia 2 months after inoculation.

Transmission from M. rhesus 32.

This animal, inoculated with culture November 18, 1926, developed bilateral granular conjunctivitis which persisted 10 months and was still progressing when the animal died of pulmonary tuberculosis on

September 10, 1927. A portion of the tarsus of the right upper lid was removed[36] on February 23, 1927, 97 days after inoculation, for transfer to *Rhesus* Nos. 53 and 54. Both developed mild but typical granular conjunctivitis which persisted for 6 months.

A second set of transfers was made on June 7, 1927, when the lesion was 201 days old, into three *rhesus* monkeys (Nos. 69, 70, and 71) and a female Japanese monkey (*Macacus speciosus*). 52 days afterwards granular conjunctivitis was present in all four animals. The histological structure of the lesion in *M. rhesus* 69 after 100 days was characteristic (Plate 29, Fig. 25).

M. rhesus 69. Left conjunctiva inoculated June 7, 1927. The inoculated lid showed a few follicles on June 22 (15 days). There was considerable granulation by July 29 (52 days), but the right lid was still smooth and pale. On Sept. 3 both lids were extensively granulated, the left somewhat more than the right, and on Sept. 15 a small piece of tissue was removed[36] from the left lid for examination and transfer (Fig. 25). The lesions continued to progress, and on Nov. 22 the lacrymal caruncle of the left eye was found to have become enlarged. This animal was seen by Drs. Knapp and Friedenwald on Jan. 26. At that time there was a minute elevation (about 1 mm. in diameter) on the sclera toward the outer corner of the eye, which had been observed about 2 weeks previously and is still present (May 25, 1928). The pupil on this side was dilated and failed to contract. The lesions are still marked on the lower lids both in the retrotarsal fold and on the tarsal conjunctiva. The lacrymal caruncle of the left eye is still enlarged.

M. rhesus 70. Duplicate of the foregoing experiment. Lesions were evident on the inoculated side within 2 weeks and were extensive after 52 days. The uninoculated eye was unaffected for several months but showed numerous follicles on Nov. 4 (150 days after inoculation of the other eye). The lesions on the left lid had by this time invaded the tarsus. The upper lids are now smooth (May 25, 1928) but some follicles are still present on the tarsal mucosa near the inner canthus.

M. rhesus 71. Inoculated at the same time as Monkeys 69 and 70 and in the same way. There was marked granulation in the retrotarsal fold of the inoculated lid after 52 days and of the uninoculated lid after 104 days. By Nov. 22 the follicles had invaded the tarsal region on both sides. On Jan. 26, when seen by Drs. Knapp and Friedenwald, the granulation of the left lid was less marked, but there was some shrinkage of the conjunctiva (beginning scar formation?). Numerous follicles are still present (May 25, 1928) on the lower lids, both in the folds and over the tarsus.

Transmission from M. rhesus 72.

Seven monkeys (*Macacus rhesus*) were inoculated on December 9, 1927, with the saline suspension of a small piece of tissue excised on

that date[36] (185 days after inoculation with cultures) from the left lid of *M. rhesus* 72. All these animals developed granular conjunctivitis.

M. rhesus 83 had developed marked granulation 32 days after inoculation, and after 81 days the right (uninoculated) eye was also involved. On May 14 the entire left tarsus was removed for transfer of the lesions to other monkeys. The right upper lid showed marked infiltration in the retrotarsal fold on May 25, 1928, and some granules on the tarsal conjunctiva. The lower right lid was covered with follicles, and the blood vessels obliterated.

M. rhesus 84 showed a very marked reaction, confined to the inoculated side, within 32 days after inoculation. The lesions are still (May 25, 1928) well marked in the retrotarsal fold and also on the tarsal conjunctiva, both upper and lower. The uninoculated eye shows only a few follicles.

M. rhesus 85 had a few follicles on the left lid when it died of tuberculosis 63 days after inoculation.

In *M. rhesus* 86 slight granulation was observed on the inoculated lid 32 days after inoculation. The other eye remained free from follicle formation for 133 days but now shows marked infiltration in the retrotarsal fold. The left tarsus was removed on May 14 for transfer of the lesions to other monkeys, but follicles are still (May 25, 1928) very marked in both canthi, and they completely cover the lower lid, both in the fold and on the tarsal conjunctiva, the blood vessels being completely obliterated.

The lesions were slow in developing in *M. rhesus* 87, appearing only after 81 days, but are now (May 25, 1928) well marked in both eyes, both in the folds and on the tarsal conjunctiva. The lower lids are similarly affected.

M. rhesus 88 showed follicle formation on the inoculated lid 32 days after inoculation and in the other eye after 133 days. Both upper lids now (May 25, 1928) show marked infiltration in the folds, and there are some follicles on the left tarsal conjunctiva.

The lesions in *M. rhesus* 89 are much less severe than in the other animals of this series.

Tables VI to VIII summarize the experimental transfers, from which the following conclusions have been deduced.

SUMMARY.

A series of transfers of the granular conjunctival lesions, directly induced by inoculation of culture, from animal to animal were made in lower monkeys and in the chimpanzee.

Six *Macacus rhesus* originally inoculated with the cultures of *Bacterium granulosis* supplied the material for the inoculation of

TABLE VI.

First Passage.

Transfer from *Macacus rhesus* No.	No. days after inoculation with culture	Transferred to *Macacus rhesus* No.	Date of transfer	Site of inoculation	Results
			1926		
5	17	21	June 29	Right upper	++ Receded in 3 mos.
"	"	22	" "	Left "	+ Transient (4 ")
"	156	2	Nov. 15	Right "	+++ Remained stationary for several mos.
"	"	3	" "	Left "	—
8	172	65	Dec. 10	Right "	—
"	"	66	" "	" "	+ Receded in 3 mos.
			1927		
"	204	44	Jan. 12	" "	++ Stationary; died of tuberculosis in 1½ mos.
"	"	45	" "	" "	++++ Progressing in both eyes when death occurred 5 mos. after inoculation
			1926		
9	37	30	Oct. 25	Both "	++++ Died under ether
"	83	38	Dec. 10	Right "	—
"	"	39	" "	" "	+ Receded in 2 mos.
			1927		
32	97	53	Feb. 23	" "	++ " " 4 " Died July 18, 1927
"	"	54	" "	" "	+ Killed because of tuberculosis after 4 mos.
"	201	69	June 7	" "	++++ Still well marked. Right eye also developed lesions
"	"	70	" "	" "	++++ Right eye also developed lesions
"	"	71	" "	" "	++++ Right eye also developed lesions
"	"	*Macacus speciosus* 1	" "	" "	+ in 53 days. Receded
72	185	83	Dec. 9	" "	++++ in 140 days. Bilateral
"	"	84	" "	" "	++++ " 32 " Right eye only slightly affected

TABLE VI—*Concluded.*

Transfer from *Macacus rhesus* No.	No. days after inoculation with culture	Transferred to *Macacus rhesus* No.	Date of transfer	Site of inoculation	Results
			1926		
72	185	85	Dec. 9	Right upper	+ Died of tuberculosis in 63 days
"	"	86	" "	" "	++++ Unilateral for 102 days. Right eye now involved
"	"	87	" "	" "	+++ in 84 days. Bilateral
"	"	88	" "	" "	+++ " 13 " . Unilateral for 100 days. Right eye now involved
"	"	89	" "	" "	+ in 115 days. Bilateral

twenty-four lower monkeys, consisting of twenty-three *Macacus rhesus* and one Japanese monkey (*Macacus speciosus*). Positive transmission results were obtained in twenty *rhesus* and one Japanese monkey. Three *rhesus* proved entirely refractory to one transfer, and the remaining one *rhesus* gave a doubtful result.

From three *rhesus* monkeys (Nos. 30, 45, and 69) in which successful transfers had been made, two chimpanzees, eighteen *rhesus*, two baboons, and one ourang-utan were inoculated. With the material from *Rhesus* 30, the transfer was highly successful in one chimpanzee, partially successful in the other chimpanzee, and in one of two *Macacus rhesus*. Material taken from *Rhesus* 45 yielded marked lesions in one *rhesus* and one baboon, and mild lesions in two *rhesus*. The results were entirely negative in eight *rhesus*, one baboon, and one ourang-utan. Two *rhesus* inoculated with material from *Rhesus* 69 also failed to react.

On the other hand, the transfer of material taken from the chimpanzee produced typical granular lesions of the conjunctiva in one *rhesus* with the chimpanzee lesion 49 days old, and in two other *rhesus* with lesions 151 days old. With the latter material a chimpanzee was also successfully inoculated. A 4th passage with material obtained from this animal was successful in four macaques and in another chimpanzee.

TABLE VII.

Second Passage.

Transfer from *Macacus rhesus* No.	No. days after inoculation	Transferred to	Date of transfer	Site of inoculation	Remarks
			1926		
30	56	Chimpanzee "Kitty"	Dec. 20	Left upper	++++ Still progressing after 8 mos.
"	"	Chimpanzee "Jimmie"	" "	" "	<+ Receded in 4 mos.
"	"	*M. rhesus* 42	" "	Right "	±
"	"	" " 43	" "	" "	+ Receded in 1 mo.
			1927		
45	63	" " 55	Mar. 15	Both "	–
"	"	" " 56	" "	" "	+ Receded in 2 mos.
"	"	Baboon 1	" "	Right "	+ in 136 days. Stationary for 6 mos.
"	"	" 2	" "	" "	+++ in 136 days. Left also involved (+). Stationary for 6 mos.
"	69	*M. rhesus* 60	" 22	Both "	Died of tuberculosis
"	"	" " 61	" "	" "	" " "
"	"	" " 34	" "	" "	– " " "
"	"	" " 35	" "	" "	–
"	"	" " 62	" "	" "	–
"	"	" " 63	" "	" "	Died of tuberculosis
"	"	" " 64	" "	" "	+ (?)
"	"	" " 65	" "	" "	–
"	"	" " 66	" "	" "	–
"	"	" " 58	" "	" "	++++ in 55 days. Died of tuberculosis in 106 days
"	"	" " 59	" "	" "	–
"	"	" " 57	" "	" "	–
69	100	" " 76	Sept. 15	" "	–
"	"	" " 77	" "	" "	–

TABLE VIII.

Third Passage.

Transfer from	No. days after inoc-ula-tion	Tranferred to	Date of transfer	Site of inoculation	Remarks
Chimpanzee “Kitty”	49	*M. rhesus* 51	*1927* Feb. 8	Left upper	++++ Progressing at time of death, 122 days after inoculation
“ “	151	Chimpanzee “Louisa”	May 20	“ “	++++ Well marked. Progressing, both eyes
“ “	“	*M. rhesus* 67	“ “	“ “	++++ Died of tuberculosis after 199 days
“ “	“	“ “ 68	“ “	“ “	+++ in 70 days. Died of tuberculosis 235 days after inoculation

Fourth Passage.

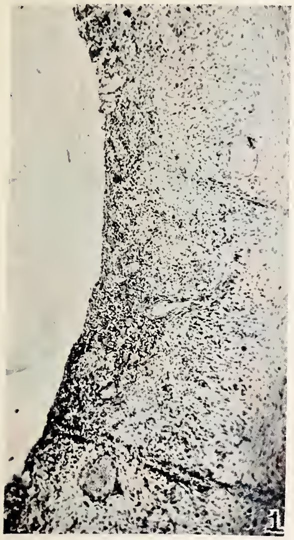
M. rhesus 67	118	*M. rhesus* 74	Sept. 15	Left upper	–
“ “ “	“	“ “ 75	“ “	“ “	–
Chimpanzee “Louisa”	146	“ “ 78	Oct. 13	“ “	+++ in 70 days. Right eye involved after 138 days. Progressing
“ “	“	“ “ 79	“ “	“ “	+++ Well marked. Progressing. Right eye involved
“ “	“	“ “ 80	“ “	“ “	+++ in 40 days. Progressing. Thickening and contraction. Probably scar tissue formation. Right eye involved
“ “	“	“ “ 81	“ “	“ “	++++ Well marked. Progressing. Right eye involved
“ “	“	“ “ 82	“ “	“ “	–

TABLE VIII—*Concluded.*

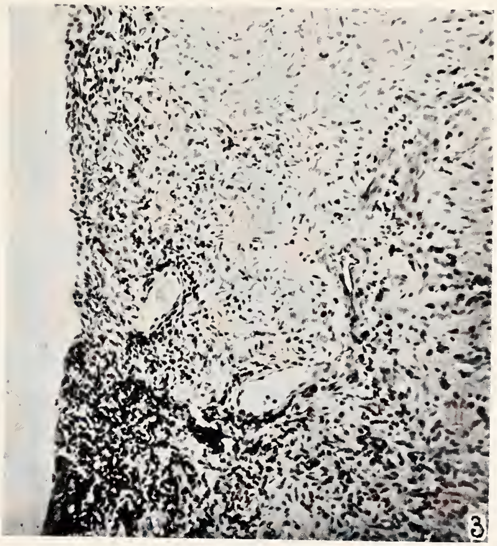
Transfer from	No. days after inoculation	Transferred to	Date of transfer	Site of inoculation	Remarks
			1927		
Chimpanzee "Louisa"	146	Chimpanzee "Venus"	Oct. 13	Left upper	+++ in 4 mos. Well marked infiltration, with obliteration of blood vessels. Right eye involved
" "	"	Chimpanzee "Adonis"	" "	" "	— Died of pneumonia 2 mos. after inoculation

The next step in the determination of the part played by *B. granulosis* in the production of chronic granular conjunctivitis in monkeys relates to the recovery in culture of the microorganism employed for inoculation.

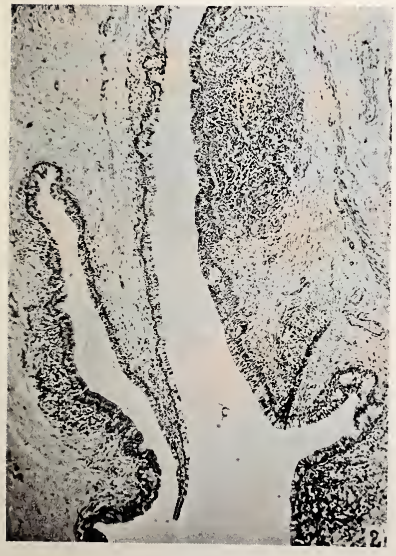
Sections of the conjunctiva of *Macacus rhesus* 45, five months after inoculation from *M. rhesus* 8. Giemsa's stain.



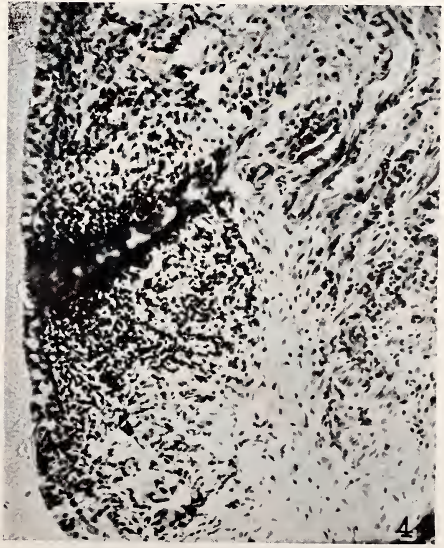
× 52.



× 156.

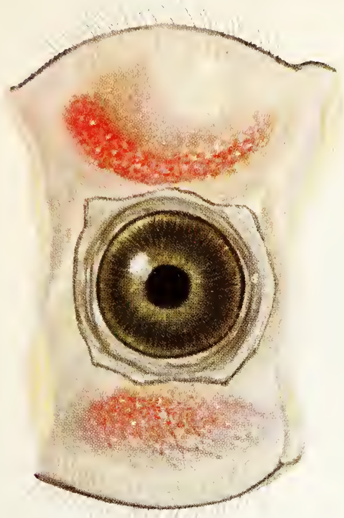


× 52.



× 156.

Conjunctivæ of *Macacus rhesus* 30, 56 days after inoculation from *Macacus rhesus* 9.



5

6

Conjunctivæ of Chimpanzee "Kitty."

7

Right—not inoculated.

8

Left—49 days after inoculation.

9

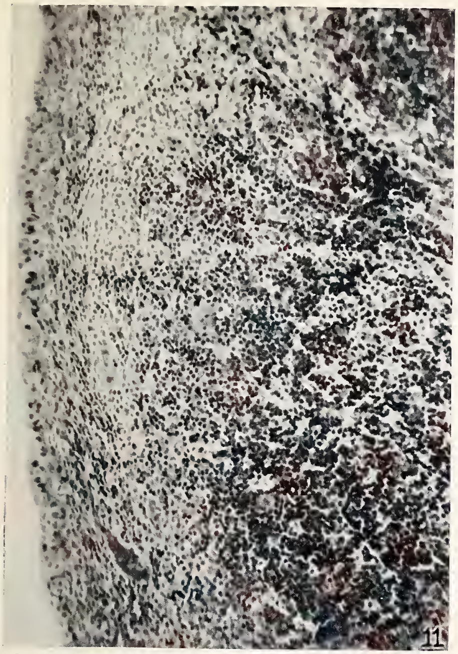
Right—98 days later, after spontaneous infection from left.

M.L.Hedge 10

Left—147 days after inoculation.

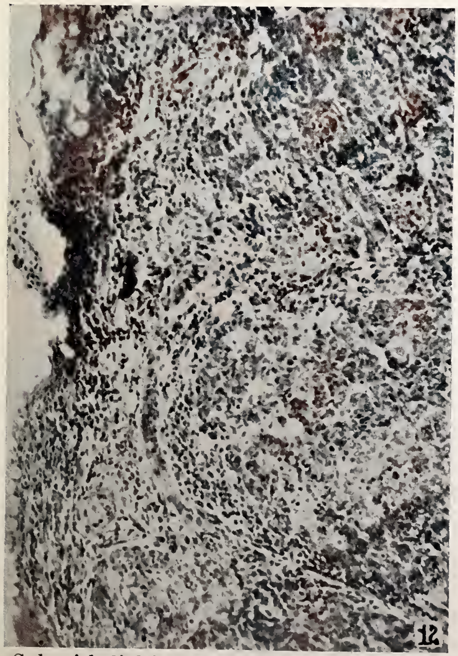
(Noguchi: Etiology of trachoma.)

Sections of the conjunctiva (left lower) of *M. rhesus* 30, 56 days after inoculation from *M. rhesus* 9. Giemsa's stain. × 156.



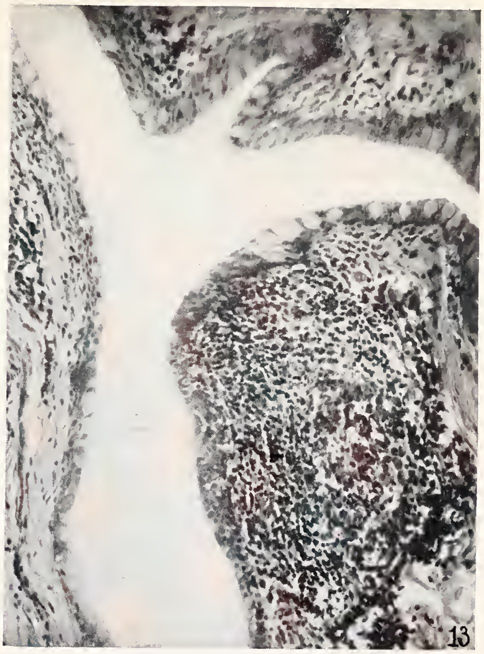
11

Subepithelial follicle, involving epithelium.



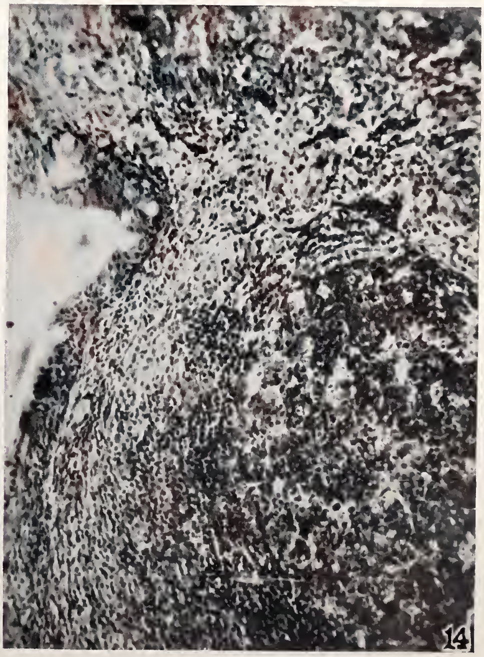
12

Subepithelial follicle, involving epithelium.



13

Papilla filled with lymphoid cells.

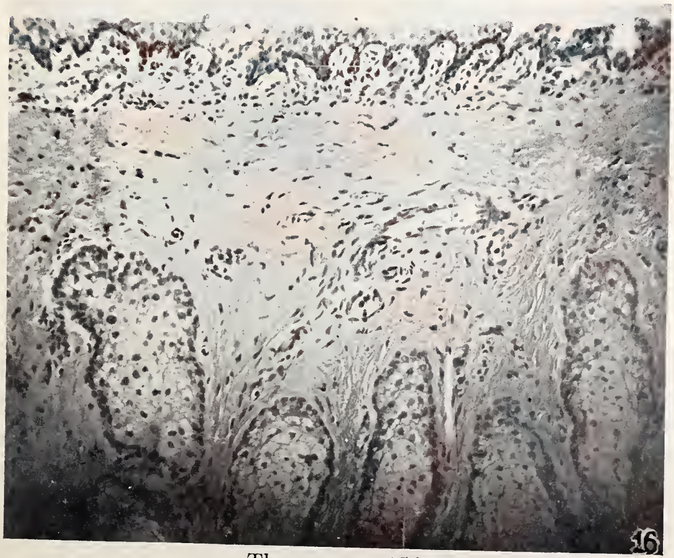


14

Follicle of laminated structure.

The conjunctiva of the normal chimpanzee. Giemsa's stain.

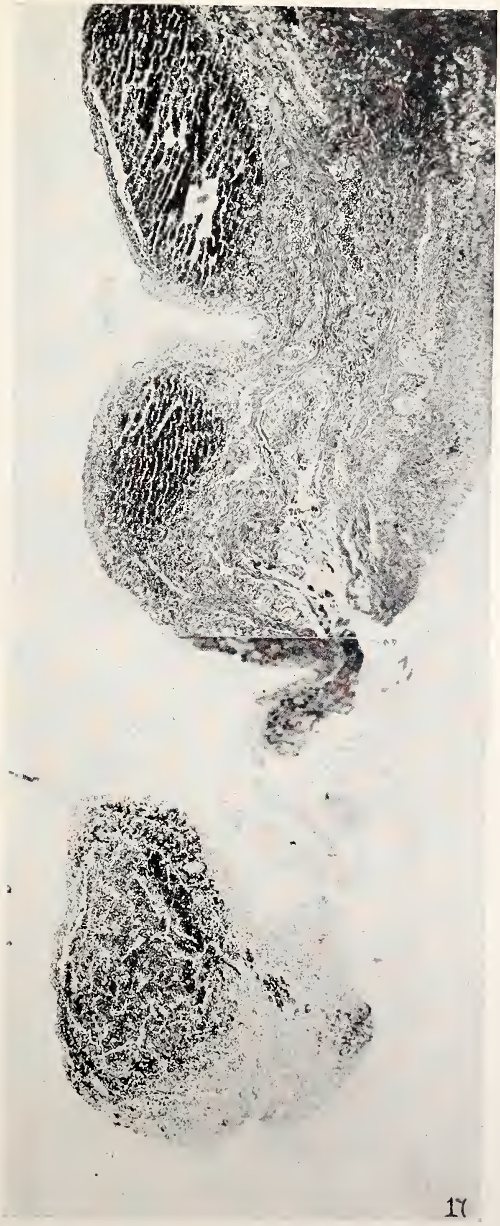
Tarsal conjunctiva, near fornix. × 52.



The same, × 156.

(Noguchi: Etiology of trachoma.)

The follicles in Chimpanzee "Kitty," 193 days after inoculation. Giemsa's stain. × 52.

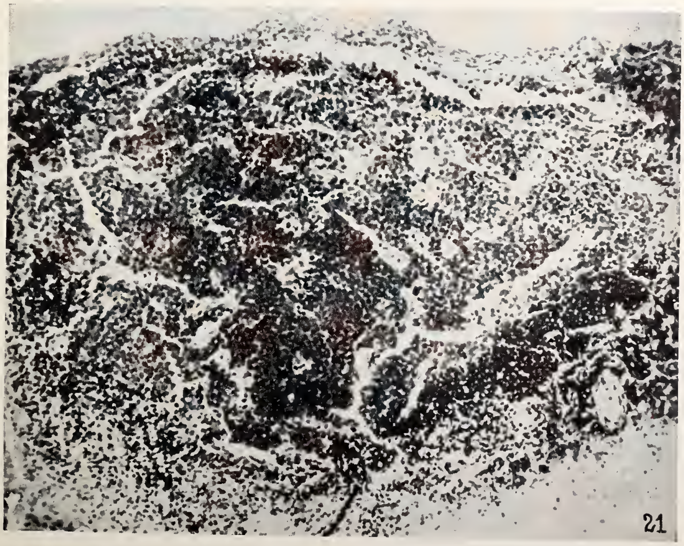


Conjunctiva of Chimpanzee "Kitty," 193 days after inoculation. Giemsa's stain, × 156, showing the invasion of the follicles by fine connective tissue fibrils.


18


19

The follicles in Chimpanzee "Kitty," 193 days after inoculation. Giemsa's stain. × 156.



(Noguchi: Etiology of trachoma.)

Giemsa's stain. Magnification × 156.

Chimpanzee "Louisa," 146 days after inoculation.

M. rhesus 67, 118 days after inoculation.

M. rhesus 67, 118 days after inoculation.

M. rhesus 69, 100 days after inoculation.

PART IV.

BACTERIUM GRANULOSIS AND THE EXPERIMENTAL AND HUMAN LESIONS.

Since the morphological features of this organism in films and sections do not serve as a means of differentiation from certain other organisms which might accidentally be present, reliance has to be placed chiefly upon its cultivation from animals used for experiment. Efforts were therefore made to recover the organism in cultures whenever suitable material was available, and it was quickly discovered that isolation was difficult because of the frequent presence of staphylococci, sarcinas, *xerosis* bacilli, organisms of the *subtilis* group, and molds. Molds were particularly troublesome, spreading over the entire surface of the media after a few days at 30°C. On several occasions, however, the materials cultured proved comparatively free from these associated organisms, and the isolation was successful. Thus, *B. granulosis* was recovered from *M. rhesus* 8, 204 days after inoculation; from *M. rhesus* 9, 37 days after inoculation; from *M. rhesus* 30, 56 days after inoculation; and from Chimpanzee "Kitty" 49 and 193 days after inoculation (Plate 31, Figs. 36–37). What is particularly important is that the bacterium was obtained not only from monkeys directly inoculated, but from those in which 1st and 2nd tissue passages had been accomplished.

In all instances the number of *B. granulosis* present was small as compared with the associated organisms. The successful method consisted in the use of several culture media simultaneously, such as freshly prepared horse blood agar plates and slants with and without the mixture of carbohydrates, leptospira medium plates containing 1 per cent agar, semisolid leptospira medium in tall tubes, and plain agar plates as controls. In the early cultivations rabbit blood was used, but later experience proved that *B. granulosis* grows poorly or not at all on rabbit blood media, especially at 37°C. A set of cultures was always placed at 37°C. for the purpose of comparing the organisms developing at this temperature with those growing at 30°C. In

initial cultures from the animal *B. granulosis* developed very slowly and growth could not be recognized for several days.

On one occasion lacrymal secretion was collected with sterile capillary pipettes from the eyes of animals showing the granular conjunctivitis (Chimpanzees "Kitty," "Jimmie," and "Marie," as well as several *rhesus* monkeys), but in no instance was *B. granulosis* recovered from such specimens.

Reinoculation of the culture obtained from Chimpanzee "Kitty" into two *rhesus* monkeys produced a mild granular conjunctivitis which persisted for about 3 months, while the inoculation of a chimpanzee with this material led to granular lesions of moderate severity still enduring for 6 months. For purposes of comparison, several *rhesus* monkeys were inoculated with staphylococci and sarcinas isolated from *M. rhesus* 5, 9, and 13, but granular lesions were not produced.

The findings just described determine unmistakably that *B. granulosis* is present in the experimental conjunctival lesions throughout the active stage of the pathologic process, since the bacillus has been recovered as early as 37 days and as late as 204 days after culture inoculation, as well as in animals of the 1st and 2nd tissue passages. The bacilli isolated from the 2nd passage lesion of a chimpanzee possessed the same pathogenic properties as the original culture obtained from Indian cases of trachoma. It would therefore appear evident that experimental granular conjunctivitis in all the monkeys was produced by *B. granulosis* alone, since the other bacterial varieties (staphylococci, sarcinas) obtained from the experimental lesions fail to produce the lesions, while *B. granulosis* not only induces the characteristic tissue changes in the conjunctivæ but persists throughout their duration and is distinctly a pathogenic organism for monkeys and apes and presumably for humans also.

B. granulosis in Human and Experimental Trachoma.

Microscopic diagnosis of acute conjunctivitis due to well known bacteria is comparatively simple, the presence of gonococci, pneumococci, streptococci, Koch-Weeks bacilli, or Morax-Axenfeld bacilli being readily recognized in a Gram-stained film preparation. The inclusion bodies of Prowazek and Halberstädter likewise offer no difficulty in recognition. No microorganism has hitherto been described, however, which is characteristic of trachoma. The Prowazek bodies,

occurring exclusively in the cytoplasm of the epithelial cells of the conjunctiva, are not specific for trachoma. They occur also in other kinds of conjunctivitis capable of being differentiated from trachoma by clinical course and ultimate effects. Moreover, they are absent from the contents of the active follicular lesions and from all parts of the conjunctivæ in cases of long standing. The minute diplobacteria described by Greeff, Frosch, and Clausen[16] as constantly present in small numbers in trachomatous lesions may have greater significance, but they appear to have been regarded as representing a phase of the Prowazek bodies. They did not grow on the culture media employed by these authors.

Microscopic search for microorganisms in the lesions of the cases of Indian trachoma was carried on simultaneously with the culture and transmission studies. Films were prepared from each case with materials expressed from the granular lesions, fixed in methyl alcohol, and stained with Giemsa's solution as well as by Gram's method. Preliminary examination of the films revealed very little, save for an occasional Gram-positive coarse *xerosis* bacillus attached to desquamated epithelial cells. Prowazek bodies could not be demonstrated. The presence of considerable amounts of amorphous material derived from the macerated cells made difficult the recognition of microorganisms present in small numbers.

After the isolation of *B. granulosis* the films were examined again minutely with a view to locating the organisms among the débris and the cells. With a definite object in view it was possible to find, in the vicinity of the small and large mononuclear cells constituting the follicle, a small number of tiny bacilli, single, paired, or in masses (Plate 30, Figs. 26–29). In clear spaces the contour of the organisms is sharply defined; some are slightly curved, many straight, often one end is thicker than the other. They are placed end to end at various angles, or lie parallel, but straight chains are seldom seen. There is a suggestion sometimes of a barred appearance, especially in the slightly curved rods with a thickened end. The organisms are 0.2 to 0.3 μ in width and 0.8 to 1.4 μ in length. They are Gram-negative and appear bluish in Giemsa preparations. They are extracellular. Occasionally a few rods resembling bacilli are seen in a ruptured Leber cell.

The striking fact about the bacilli is that they are present in extremely small numbers, and prolonged and careful search is required to find them. They do not stain as intensely as the culture forms of

B. granulosis, and they appear somewhat thinner. The scarcity of the organisms in smears, however, was indicated also by the culture results.

The bacilli have not yet been sought systematically in sections of tissue, but they have been found in one of several excised tarsi furnished by Dr. Richards (Plate 30, Figs. 30–31).

The film preparations (Plate 31, Figs. 32–33) and sections (Plate 31, Figs. 34–35) from inoculated animals yielded results agreeing with those obtained with the human material.

Comparison of Human and Experimental Lesions.

Numerous careful studies of the histological structure of human trachoma have been made by previous investigators, and all agree in this, namely, that trachoma differs from all other kinds of conjunctivitis by the final sclerosis of the conjunctival mucosa, the formation of scar tissue, and entropion of the affected lids.[16, 23, 38–44] While it would be highly informing to study human tissues at different stages of the disease, the opportunity to obtain specimens in the early stage is precluded, because at this period operative intervention is not required. Moreover, clinical diagnosis is difficult until the appearance of scar tissue. It is only by reason of the small number of transmission tests in human volunteers that we possess reliable information as to the contagiosity, period of incubation, varieties of early clinical manifestations, and histological changes of human trachoma.

According to Addario,[45] who performed three such tests in Italy, the earliest symptoms appear in 4 to 7 days and include hyperemia of the inoculated conjunc-

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[38] Addario, C., *Arch. Augenheilk.*, 1900, xli, 20; *Arch. ottal.*, 1906–07, xiv, 65, 270.

[39] Collins, T., *Brit. Med. J.*, 1909, ii, 973.

[40] Fuchs, E., Lehrbuch der Augenheilkunde, Leipsic and Vienna, 6th edition, 1897.

[41] de Schweinitz, G. E., Diseases of the conjunctiva, Philadelphia and London, 1924, 236–243.

[42] Morax, V., Pathologie oculaire, Paris, 1921, 93–101.

[43] Clark, T., and Schereschewsky, J. W., *Treas. Dept., U. S. P. H.*, 1907.

[44] Solovieff, P., *Arch. Inst. Pasteur Afrique Nord*, 1921, i, 388.

[45] Addario, C., *Arch. ottal.*, 1906–07, xiv, 321.

tiva and lacrymation and a pricking sensation. These give place to tumefaction of the lids, and within 11 to 29 days grayish granules appear on the conjunctiva along the upper border of the tarsus. Mucopurulent secretions may be present at first but diminish in 2 to 3 weeks. The catarrhal condition passes into a subacute granular conjunctivitis which involves not only the inoculated lid but also the normal one. Papillary hyperplasia, evident by a congested velvety appearance or roughness of the conjunctival surface, ensues within 45 days after inoculation. At this stage the tarsal conjunctiva becomes diffusely affected and shows more or less prominent follicles, the so called sago granules. Histological study shows lymphocytic infiltration and follicle formation in the adenoid layer involving the epithelium, which proliferates. Where the follicles have attained large size the epithelium over them is forced outwards in such a way as to flatten the epithelial cells, or it may even be ruptured. The epithelial layer, however, is not the primary site of change but is secondarily invaded through cellular infiltration of the subepithelial layer.

The conditions described last for many months or years, rupture and evacuation of the follicles occurring from time to time. As a result of this ulcerative process fibrous elements form throughout the subepithelial layer, and the epithelium becomes thickened and in places completely flattened. A large number of fibroblasts and connective tissue fibers form, leading to cicatrization of the conjunctival mucosa and submucosa and production of permanent scars, in course of which the lymphocytic cells and follicles are in time replaced with scar tissue.

The trachoma follicles or granules have been the subject of much study, but nothing of specific nature, except their tendency to ulcerate, has been determined concerning them. The follicles usually consist of an outer layer of small lymphocytes and a central portion of larger, rather tightly packed mononuclear cells resembling the germinal center of lymphatic nodules. In the inner part the nuclei are vesicular and the cytoplasm more abundant and paler staining than that of the small lymphocytes. The follicle may or may not be enclosed by a capsule. The presence of numerous mitotic figures among the large cells indicates active proliferation, and the large cells containing deeply or lightly staining round or oval bodies of various sizes within the swollen and thinned protoplasm are taken to show either a state of toxic degeneration or phagocytosis. The cells known as Leber's granule cells (*Körperchenzellen*) are by no means characteristic of trachoma, as pointed out by Leber himself.[46] Mast cells may be intermingled with other cells in the follicle but occur more often in the interstitial spaces of the connective tissue.

The histological appearances which are observed in the excised tarsal tissues from cases of trachoma occurring among the American Indians agree with those described above as present in similar materials from recognized cases of trachoma elsewhere.

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[46] Leber, T., *Ber. Versamml. Ophth. Ges., 1896, Wiesbaden*, 1897, xxv, 156.

Experimental Lesions in Macacus rhesus and Chimpanzee.—The earliest lesion studied was that on the upper lid of *M. rhesus* 5, 17 days after the inoculation of culture. Well advanced infiltration of lymphocytes and large mononuclear cells in the subepithelial layer existed along with few polymorphonuclear leucocytes. Here and there small masses of lymphoid cells were observed pushing out the epithelium. 156 days after inoculation the entire tarsus was removed from this animal. The granular appearance had become much more pronounced and extended to the tarsal region. As the photographs show (Plate 18), the infiltration and follicle formation resemble closely the conditions present in human trachoma.

Even more pronounced were the lesions removed from another animal (Monkey 9) 37 days after the inoculation of culture (Plate 20). As had been observed clinically, numerous large, well developed follicles were present, and these showed structures like those present in the human lesions from which the culture had been obtained. The follicles occupied chiefly the upper border of the tarsus and the region of the cul-de-sac. Where they were most highly developed, the epithelial layer was raised and infiltrated with lymphoid cells. Near the apices the epithelial layer was discontinuous, and along the lateral surface of the prominent follicles especially there were many goblet cells. The follicles were sometimes surrounded by a capsule. The tarsal conjunctiva was much infiltrated, chiefly diffusely with small foci in aggregate or follicle form. This destruction in histological process is undoubtedly due, as may be inferred from examination of the normal conjunctiva of *Macacus rhesus*, to the little loose subepithelial layer or adenoid tissue in the monkey compared to man. The lesions here considered arose by spontaneous transmission from the inoculated eye and not by direct inoculation of culture.

M. rhesus 30, inoculated with an emulsion of the excised tissue from *M. rhesus* 9, developed well marked granular conjunctivitis from which tissues were excised 56 days after inoculation. The histological lesions (Plate 24, Figs. 11–14) were identical with, but more extensive than, those of Monkey 9. The lower conjunctiva, which had not been inoculated, showed the same kind of lesions as the inoculated upper lid, in both of which occurred large follicles of typical structure, that is, some with small lymphocytes along the periphery and large mono-

nuclears in the central areas, some definitely encapsulated. No cell necrosis existed, but on the contrary numerous mitotic figures were seen.

The lesions in the chimpanzees (Plates 26–28, Plate 29, Fig. 22) showed the same histological structures as those just described in lower monkeys.

Scar Formation.—Monkey 8, in which inoculation of culture had given rise to granular conjunctivitis similar to that of Monkey 5 but progressing more rapidly, showed after nearly 7 months fine streaks of scar tissue in the upper conjunctivæ. The tarsus was thickened, congested, and presented an uneven surface. Histological study of tissue removed 234 days after inoculation, revealed very little infiltration and only remnants of diminishing follicles (Plate 19, Figs. 28–29). The epithelium consisted of flattened cells, beneath which increase of connective tissue cells and fibrous elements was present. In other words, a well marked sclerosis of the tarsal conjunctiva had developed. Similarly, scars occurred in Monkeys 9, 32, and 80 and in Chimpanzee "Kitty," 10 to 12 months after inoculation.

The descriptions which have been given of the microscopic appearances of the conjunctival tissues of the inoculated monkeys leave no doubt of close similarity to the histological characters found in cases of human trachoma. In both man and monkey there occur diffuse and nodular subepithelial lymphocytic infiltration. The large follicles do not appear in the tarsal region in the monkey because this region, unlike that in man, is almost devoid of the adenoid layer in which the large follicles develop. In the monkey the epithelium is gradually invaded by lymphocytes, and through the pressure of these cells and the enlarging follicles the layer gives way. The follicular contents are thus evacuated, following which fibrosis or scar formation takes place.

Although unable as a rule to produce long standing chronic affection with final destruction of the conjunctiva, pannus, and scar tissue formation, Hess and Römer,[20] Morax,[21] Nicolle, Cuénod, and Blaizot,[47] Bertarelli and Cecchetto,[30] and others who transmitted trachoma to monkeys regard the experimental lesions as histologically identical with those of the human disease. It is impressive to observe how closely the results obtained by direct transmission with human

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[47] Nicolle, C., Cuénod, A., and Blaizot, L., *Compt. rend. Acad.*, 1912, clv, 241; clvi, 1177; *Arch. Inst. Pasteur Tunis*, 1911, iii, 185.

materials parallel those obtained in the present investigation by means of cultures. The percentage of successful transmissions by direct inoculation from human lesions was about 50 in chimpanzees (one chimpanzee in eight inoculated by Morax, and five of five inoculated by Nicolle and his collaborators), and about 25 in baboons (41 baboons having been inoculated by various workers) and in *Macacus* monkeys. Inoculation of ourang-utans has usually failed. Direct transmission from animal to animal as reported has given inconstant and often negative results, and no one except Nicolle and his collaborators seems to have maintained a strain beyond the 2nd, or at most the 3rd, passage.

We may repeat here that the culture employed in the present study failed to infect an ourang-utan but excited characteristic lesions, either directly, or by animal passage, in chimpanzees and *Macacus* monkeys and also in the baboon. Finally, it should be emphasized that one experiment started with the culture is now in its 5th series (culture to *rhesus*, *rhesus* to *rhesus*, *rhesus* to chimpanzee, chimpanzee to chimpanzee, and chimpanzee to *rhesus*).

SUMMARY.

The experimental chronic granular conjunctivitis first induced in *Macacus rhesus* with pure cultures of *Bacterium granulosis*, has been transferred by direct tissue passage to the chimpanzee, baboon, and to other *Macacus rhesus* through at least 4 successive passages.

This experimental chronic granular conjunctivitis preserved the clinical and histological characters throughout the several passages, and the lesions have been shown to be infective as early as 17 and as late as 204 days after the original culture inoculation.

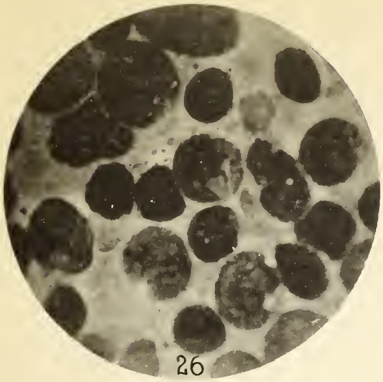
Bacterium granulosis can be recovered from the inoculated animals and has been found in microscopic specimens of human and monkey tissues. The great difficulty of its recovery in culture and its demonstration in sections of tissues and in films is not necessarily an indication of its absence from the lesions. The methods employed to recover or to find the organism may not be the most favorable to be discovered.

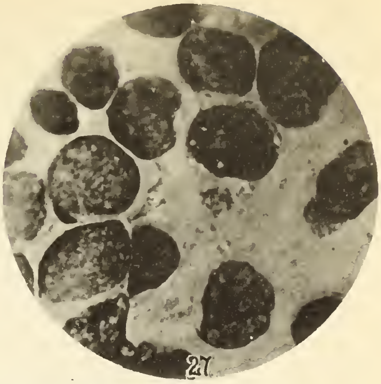
No other microorganism obtained from the human cases of trachoma produces in animals effects comparable to those induced by *Bacterium granulosis*. In the absence, therefore, of indications to the contrary, we may consider that in *Bacterium granulosis* we have the inciting microorganism of trachoma in man and its equivalent, granular conjunctivitis in monkeys.

ADDENDUM.

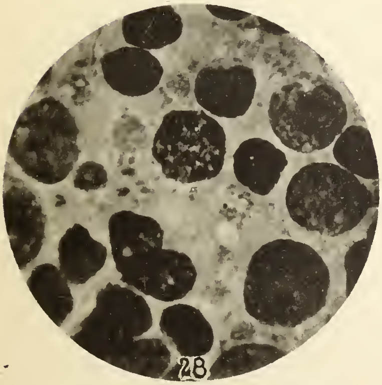
Dr. Noguchi left New York on October 22, 1927, for Accra, Gold Coast, West Africa. In his absence the observations on inoculated monkeys and the further transfer of the trachomatous lesions of the monkeys were carried out, according to his plans, by his assistants. Moreover, on several occasions during his absence, the monkeys were examined by Drs. F. I. Proctor, P. Richards, A. Knapp, J. S. Friedenwald, M. Cohen, and W. C. Posey, and the results of these examinations are recorded in the individual protocols. The last examination was made on May 25, 1928.

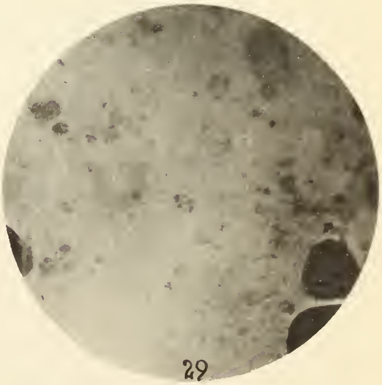
B. granulosis in materials from human trachoma.


26

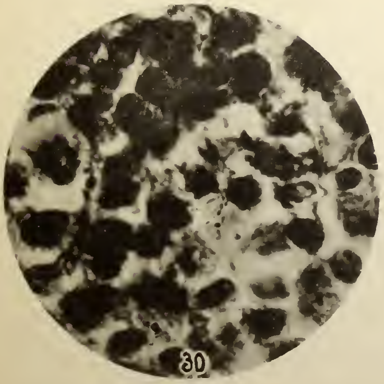

27

Film, Giemsa's stain. ×1000.

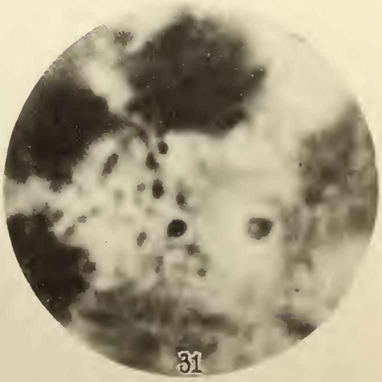

28


29

Film, Giemsa's stain. ×1000.


30

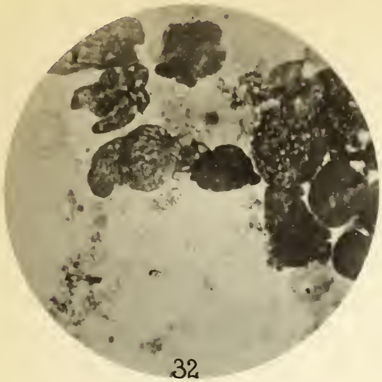
×1000


31

×2200

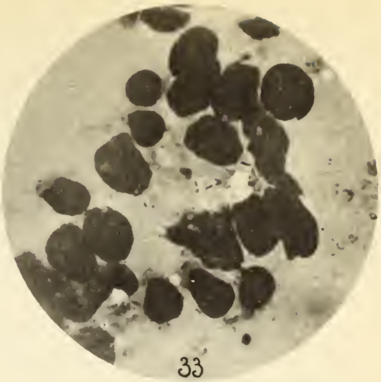
Section of tarsus. Giemsa's stain.

B. granulosis in experimental materials.



32

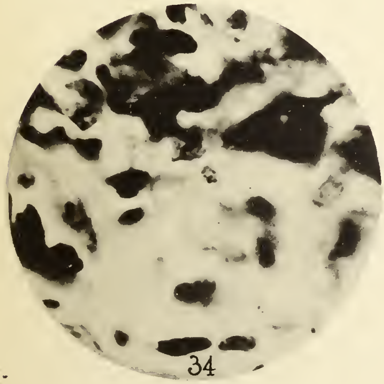
Film, *M. rhesus* 32.



33

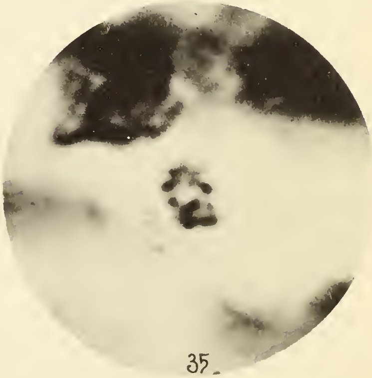
Film, Chimpanzee "Kitty."

Giemsa's stain. ×1000.



34

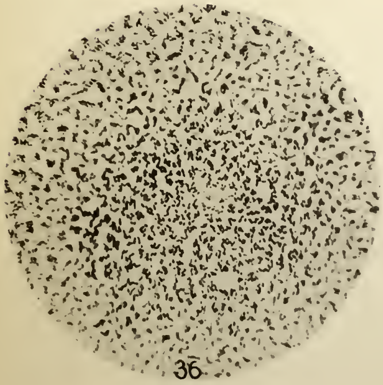
×1000.



35

×2200.

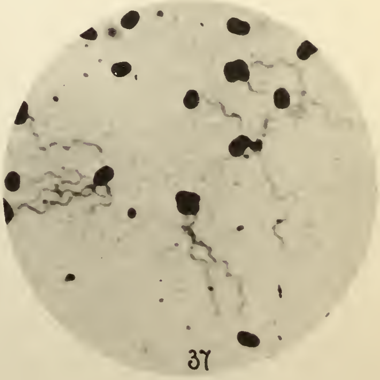
Section, Chimpanzee "Kitty." Giemsa's stain.



36

×1000

Gram's stain, counterstained
with fuchsin.



37

×2200

Zettnow-Fontana stain.

Culture isolated from Chimpanzee "Kitty."